

EX LIBRIS

ROBERT L. PHILLIPS, M.D.



Digitized by the Internet Archive
in 2025

THE HARVEY SOCIETY

**THE
HARVEY LECTURES**
Delivered under the auspices of
**THE HARVEY SOCIETY
OF NEW YORK**

Previously Published

<i>FIRST SERIES.....</i>	<i>1905-1906</i>
<i>SECOND SERIES.....</i>	<i>1906-1907</i>
<i>THIRD SERIES.....</i>	<i>1907-1908</i>
<i>FOURTH SERIES.....</i>	<i>1908-1909</i>
<i>FIFTH SERIES.....</i>	<i>1909-1910</i>
<i>SIXTH SERIES.....</i>	<i>1910-1911</i>
<i>SEVENTH SERIES...</i>	<i>1911-1912</i>
<i>EIGHTH SERIES.....</i>	<i>1912-1913</i>
<i>NINTH SERIES.....</i>	<i>1913-1914</i>
<i>TENTH SERIES.....</i>	<i>1914-1915</i>
<i>ELEVENTH SERIES..</i>	<i>1915-1916</i>
<i>TWELFTH SERIES...</i>	<i>1916-1917</i>
<i>THIRTEENTH SERIES</i>	<i>1917-1918</i>
<i>FOURTEENTH SERIES</i>	<i>1918-1919</i>
<i>FIFTEENTH SERIES.</i>	<i>1919-1920</i>
<i>SIXTEENTH SERIES.</i>	<i>1920-1921</i>
<i>SEVENTEENTH</i>	<i>SERIES 1921-1922</i>
<i>EIGHTEENTH</i>	<i>SERIES 1922-1923</i>
<i>NINETEENTH</i>	<i>SERIES 1923-1924</i>

“The Harvey Society deserves the thanks of the profession at large for having organized such a series and for having made it possible for all medical readers to share the profit of the undertaking.”

—*Medical Record, New York.*

J. B. LIPPINCOTT COMPANY
Publishers Philadelphia

THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF

THE HARVEY SOCIETY OF NEW YORK

UNDER THE PATRONAGE OF THE NEW YORK ACADEMY OF MEDICINE

1923-1924

BY

DR. A. BIEDE
DR. WALTER R. BLOOR
DR. WALTER A. JACOBS
DR. DAVID MARINE

DR. EVARTS A. GRAHAM
DR. JOHN J. ABEL
DR. HERBERT M. EVANS
DR. LUDWIG ASCHOFF

SERIES XIX

~~8115~~

PHILADELPHIA, LONDON, MONTREAL
J. B. LIPPINCOTT COMPANY

COPYRIGHT, 1925
By J. B. LIPPINCOTT COMPANY

PRINTED AT THE WASHINGTON SQUARE PRESS
BY J. B. LIPPINCOTT COMPANY
PHILADELPHIA, U. S. A.

PREFACE

THE nineteenth series of Harvey Society lectures presented in this volume offers again to those interested in the progress of medicine and cognate sciences, a collection of papers by investigators eminently able to speak authoritatively on their respective subjects. To these busy workers who have devoted the time and effort necessary for the preparation and presentation of lectures, the Society expresses its very genuine appreciation.

It is just twenty years since a group of men at the invitation of Dr. Graham Lusk met to form a Society whose object should be the diffusion of scientific knowledge. The present volume is published with the belief that it will contribute like its eighteen predecessors to the achievement of that object.

To Williams and Wilkins Company, Paul B. Hoeber, and the Physiatrie Institute, the Editor is indebted for permission to republish the lectures of Doctor Marine, Professor Aschoff and Professor Bloor.

GEORGE M. MACKENZIE, *Secretary.*

May, 1925.

THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE
MEDICAL SCIENCES

CONSTITUTION

I.

This Society shall be named the Harvey Society.

II.

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III.

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the society and who are neither active nor associate members. Associate and honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

CONSTITUTION

IV.

The management of the Society shall be vested in an executive committee to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V.

The Annual meeting of the Society shall be held soon after the concluding lecture of the course given during the year, at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all the meetings *ten* members shall constitute a quorum.

VI.

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification of the members in writing.

OFFICERS OF THE HARVEY SOCIETY

OFFICERS

1924-1925

EUGENE F. DuBois, President

PEYTON ROUS, Vice-President

GEORGE M. MACKENZIE, Secretary

CHARLES C. LIEB, Treasurer

COUNCIL

1924-1925

GRAHAM LUSK

RUFUS COLE

HAVEN EMERSON

WILLIAM H. PARK

The Officers Ex-Officio

ACTIVE MEMBERS

DR. F. M. ALLEN	DR. R. BURTON-OPITZ
DR. H. A. AMOSS	DR. ALEXIS CARREL
DR. C. H. ANDREWES	DR. RUSSELL L. CECIL
DR. DANA W. ATCHLEY	DR. JOHN W. CHURCHMAN
DR. HUGH AUCHINCLOSS	DR. A. F. COCA
DR. JOHN AUER	DR. ALFRED E. COHN
DR. J. HAROLD AUSTIN	DR. RUFUS COLE
DR. O. T. AVERY	DR. ROBERT A. COOKE
DR. GEORGE BAEHR	DR. C. B. COULTER
DR. C. V. BAILEY	DR. E. V. COWDRY
DR. HORACE S. BALDWIN	DR. GLENN E. CULLEN
DR. F. W. BANCROFT	DR. H. W. DAHL
DR. W. H. BARBER	DR. H. D. DAKIN
DR. D. P. BARR	DR. A. R. DOCHEZ
DR. EMIL BAUMAN	DR. DOUGLAS DRURY
DR. LOUIS BAUMAN	DR. E. F. DuBOIS
DR. W. W. BEATTIE	DR. C. B. DUNLAP
MISS ETHEL M. BENEDICT	DR. HALBERT L. DUNN
DR. S. R. BENEDICT	DR. A. H. EBELING
DR. CARL H. BINGER	DR. WALTER H. EDDY
DR. FRANCIS G. BLAKE	DR. D. J. EDWARDS
DR. N. F. BLAU	DR. CARY EGGLESTON
DR. CHARLES F. BOLDUAN	DR. ROBERT ELMAN
DR. RALPH H. BOOTS	DR. W. J. ELSER
DR. ARNOLD BRANCH	DR. A. ELWYN
DR. J. J. BRONFENBRENNER	DR. HAVEN EMERSON
DR. HARLOW BROOKS	DR. EPHRAIM M. EWING
DR. G. O. BROWN	DR. JAMES EWING
DR. GEORGE R. BROW	DR. L. W. FAMULENER
DR. WADE H. BROWN	DR. LLOYD D. FELTON
DR. LEO BUERGER	DR. J. S. FERGUSON
DR. F. C. BULLOCK	DR. CYRUS W. FIELD

ACTIVE MEMBERS—*Continued*

DR. MORRIS S. FINE	DR. CHARLES KRUMWIEDE
DR. SIMON FLEXNER	DR. WILLIAM S. LADD
DR. NELLIS B. FOSTER	DR. R. V. LAMAR
DR. ALEXANDER FRASER	DR. ROBERT A. LAMBERT
DR. FRANCIS R. FRASER	DR. KARL LANDSTEINER
DR. FREDERICK L. GATES	DR. NILS P. LARSEN
DR. F. C. GEPHART	DR. FREDERIC S. LEE
DR. ALEXANDER O. GETTLER	DR. E. S. L'ESPERANCE
DR. H. R. GEYELIN	DR. P. A. LEVENE
DR. W. J. GIES	DR. ISAAC LEVIN
DR. W. C. VON GLAHN	DR. ROBERT L. LEVY
DR. ROSS GOLDEN	DR. E. LIBMAN
DR. S. GOLDSCHMIDT	DR. C. C. LIEB
DR. FREDERICK GOODRIDGE	DR. G. C. LINDER
DR. ISADORE GREENWALD	DR. ROBERT F. LOEB
DR. F. M. HANGER, JR.	DR. WARFIELD T. LONGCOPE
DR. GEORGE HARROP	DR. CHRISTEN LUNDSGAARD
DR. BENJAMIN HARROW	DR. GRAHAM LUSK
DR. ALBERT R. HASTINGS	DR. CLARA J. LYNCH
DR. T. W. HASTINGS	DR. P. H. DEKRUIF
DR. ROBERT A. HATCHER	DR. GERTRUDE F. McCANN
DR. M. HEIDELBERGER	DR. W. S. McCANN
DR. ALFRED F. HESS	DR. F. H. MCCRUDDEN
DR. J. G. HOPKINS	DR. JOHN F. McINTOSH
DR. PAUL E. HOWE	DR. F. C. McLEAN
DR. G. S. HUNTINGTON	DR. P. D. McMASTER
DR. R. G. HUSSEY	DR. W. J. McNEAL
DR. HOLMES C. JACKSON	DR. W. G. MACCALLUM
DR. W. A. JACOBS	DR. GEORGE M. MACKENZIE
DR. JAMES W. JOBLING	DR. A. R. MANDEL
PROF. W. C. JOHNSON	DR. JOHN A. MANDEL
DR. DONALD R. JOSEPH	DR. F. S. MANDELBAUM
DR. D. M. KAPLAN	DR. HUBERT MANN
DR. JOHN A. KILLIAN	DR. W. H. MANWARING
DR. I. S. KLEINER	DR. DAVID MARINE

ACTIVE MEMBERS—*Continued*

DR. HUGH MORGAN	DR. HARRY PLOTZ
DR. ADOLPH MEYER	DR. LEO M. POWELL
DR. G. M. MEYER	DR. P. V. PREWETT
DR. C. P. MILLER	DR. IDA W. PRITCHETT
DR. EDGAR G. MILLER	DR. FREDERICK PRIME
DR. L. S. MILNE	DR. BRET RATNER
DR. C. V. MORRILL	DR. A. N. RICHARDS
DR. H. O. MOSENTHAL	DR. HENRY B. RICHARDSON
DR. J. R. MURLIN	DR. A. I. RINGER
DR. J. B. MURPHY	DR. MICHAEL RINGER
DR. HENRY A. MURRAY	DR. THOMAS M. RIVERS
DR. VICTOR C. MYERS	DR. C. G. ROBINSON
DR. WARO NAKAHARA	DR. IDA PAULINE ROLF
DR. JAMES M. NEIL	DR. G. L. ROHDENBURG
DR. FREDERICK M. NICHOLSON	DR. ALFRED W. ROMER
DR. W. C. NOBLE	DR. A. R. ROSE
DR. HIDEYO NOGUCHI	DR. M. A. ROTHSCHILD
DR. CHARLES NORRIS	DR. PEYTON ROUS
DR. P. L. DU NOUY	DR. HAROLD A. SALVESEN
DR. HORST OERTEL	DR. H. VON SCHULTE
DR. PETER K. OLITSKY	DR. OTTO H. SCHULTZE
DR. EUGENE L. OPIE	DR. E. L. SCOTT
DR. B. S. OPPENHEIMER	DR. H. D. SENIOR
DR. REUBEN OTTENBERG	DR. H. F. SHATTUCK
DR. WALTER W. PALMER	DR. C. P. SHERWIN
DR. A. M. PAPPENHEIMER	DR. GERALD S. SHIBLEY
DR. WILLIAM H. PARK	DR. M. J. SITTENFIELD
MRS. JULIA PARKER	DR. BERTRAM G. SMITH
DR. F. W. PEABODY	DR. W. C. STADIE
DR. LOUISE PEARCE	DR. FRANKLIN A. STEVENS
DR. E. J. PELLINI	DR. HAROLD J. STEWART
DR. WILDER G. PENFIELD	DR. E. G. STILLMAN
DR. J. P. PETERS	DR. EDGAR STILLMAN
DR. M. HORTENSE PFALTZ	DR. ARTHUR P. STOUT
DR. F. H. PIKE	DR. I. STRAUSS

ACTIVE MEMBERS—*Continued*

DR. OLIVER S. STRONG	DR. WILLIAM H. WALKER
DR. HOMER F. SWIFT	DR. C. B. WALLACE
DR. DOUGLAS SYMMERS	DR. LESLIE T. WEBSTER
DR. B. T. TERRY	DR. RANDOLPH WEST
DR. WILLIAM S. TILLET	DR. J. S. WHEELWRIGHT
DR. J. C. TORREY	DR. C. G. WIGGERS
DR. JAMES D. TRASK, JR.	DR. ANNA WILLIAMS
DR. E. UHLENHUTH	DR. H. B. WILLIAMS
DR. C. M. VAN ALLEN	DR. ROBERT J. WILSON
DR. DONALD D. VAN SLYKE	DR. WILLIAM J. WOGLOM
DR. KARL M. VOGEL	DR. MARTHA WOLLSTEIN
DR. AUGUSTUS WADSWORTH	DR. JONATHAN WRIGHT
DR. A. J. WAKEMAN	DR. HANS ZINSSER

ASSOCIATE MEMBERS

DR. T. J. ABBOTT	DR. R. J. CARLISLE
DR. F. H. ALBEE	DR. HERBERT S. CARTER
DR. H. L. ALEXANDER	DR. L. CASAMAJOR
DR. WALTER P. ANDERTON	DR. ARTHUR F. CHACE
DR. R. T. ATKINS	DR. THOMAS H. CHERRY
DR. HAROLD BAILEY	DR. H. T. CHICKERING
DR. CLARENCE G. BANDLER	DR. F. MORRIS CLASS
DR. ALVAN R. BARACH	DR. MATHER CLEVELAND
DR. DAVID N. BARROWS	DR. CORNELIUS G. COAKLEY
DR. F. H. BARTLETT	DR. MARTIN COHEN
DR. WALTER A. BASTEDO	DR. L. G. COLE
DR. EDWIN BEER	DR. WARREN COLEMAN
DR. CONRAD BERENS	DR. WILLIAM B. COLEY
DR. JOSEPH A. BLAKE	DR. CHARLES F. COLLINS
DR. S. R. BLATTEIS	DR. LEWIS A. CONNER
DR. GEORGE BLUMER	DR. G. W. CRARY
DR. ERNEST BOAS	DR. EDWARD CUSSLER
DR. RICHARD W. BOLLING	DR. CHARLES L. DANA
DR. A. BOOKMAN	DR. THOMAS DARLINGTON
DR. S. BRADBURY	DR. WILLIAM DARRACH
DR. STANLEY BRADY	DR. D. BRYSON DELAVAN
DR. J. W. BRANNAN	DR. ED. B. DENCH
DR. A. BRASLAU	DR. BLAKE F. DONALDSON
DR. J. BRETTAUER	DR. W. K. DRAPER
DR. GEORGE E. BREWER	DR. ALEXANDER DUANE
DR. NATHAN E. BRILL	DR. THEODORE DUNHAM
DR. SAMUEL A. BROWN	DR. MAX EINHORN
DR. JESSE G. M. BULLOWA	DR. C. A. ELSBERG
DR. CARL BURDICK	DR. A. A. EPSTEIN
DR. SIDNEY R. BURNAP	DR. SEWARD ERDMAN
DR. GLENTWORTH R. BUTLER	DR. EVAN M. EVANS
DR. W. E. CALDWELL	DR. S. M. EVANS
DR. WILLIAM F. CAMPBELL	DR. LILLIAN K. P. FARRAR

ASSOCIATE MEMBERS—*Continued*

DR. MAURICE FISHBERG	DR. SERGIUS INGERMAN
DR. ROLFE FLOYD	DR. LEOPOLD JACQUES
DR. R. G. FREEMAN	DR. GEORGE W. JACOBY
DR. WOLFF FREUNDENTHAL	DR. RALPH JACOBY
DR. E. D. FRIEDMAN	DR. WALTER B. JAMES
DR. LEWIS D. FRISSELL	DR. S. E. JELLIFFE
DR. H. DAWSON FURNISS	DR. L. KAST
DR. LESLIE T. GAGER	DR. JACOB KAUFMANN
DR. CHARLES GOODMAN	DR. F. L. KEAYS
DR. S. S. GOLDWATER	DR. FOSTER KENNEDY
DR. MALCOLM GOODRIDGE	DR. C. G. KERLEY
DR. RODERICK V. GRACE	DR. LEO KESSEL
DR. N. W. GREEN	DR. E. L. KEYES, JR.
DR. J. C. GREENWAY	DR. R. A. KINSELLA
DR. MENAS S. GREGORY	DR. GEORGE H. KIRBY
DR. ROBERT H. HALSEY	DR. ARNOLD KNAPP
DR. JOHN M. HANFORD	DR. LINNAEUS LA FETRA
DR. J. A. HARRAR	DR. ALBERT R. LAMB
DR. T. S. HART	DR. ADRIAN V. S. LAMBERT
DR. JOHN A. HARTWELL	DR. ALEXANDER LAMBERT
DR. H. A. HAUBOLD	DR. S. W. LAMBERT
DR. ROYAL S. HAYNES	DR. BOLESLAW LAPOWSKI
DR. EDSON B. HECK	DR. BERTON LATTIN
DR. HENRY HEIMAN	DR. B. J. LEE
DR. W. W. HERRICK	DR. JEROME S. LEOPOLD
DR. C. GORDON HEYD	DR. L. T. LEWALD
DR. WALTER HIGHMAN	DR. RICHARD LEWISON
DR. J. MORLEY HITZROT	DR. ASA L. LINCOLN
DR. FREDERICK C. HOLDEN	DR. ELI LONG
DR. H. A. HOUGHTON	DR. WILLIAM C. LUSK
DR. HUBERT S. HOWE	DR. HENRY H. LYLE
DR. FRANCIS HUBER	DR. D. HUNTER McALPIN
DR. E. LIVINGSTON HUNT	DR. KENNETH McALPIN
DR. WOODS HUTCHINSON	DR. JOSEPH F. MCCARTHY
DR. HAROLD T. HYMAN	DR. JOHN MCCOY
DR. H. M. IMBODEN	DR. D. B. MACKENZIE

ASSOCIATE MEMBERS—*Continued*

DR. JOHN T. MACURDY	DR. JAMES PEDERSON
DR. MORRIS MANGES	DR. FREDERICK PETERSON
DR. GEORGE MANNHEIMER	DR. RICHARD N. PIERSON
DR. WALTON MARTIN	DR. SIGISMUND POLLITZER
DR. HOWARD H. MASON	DR. EUGENE H. POOL
DR. FRANK S. MEARA	DR. EDWARD L. PRATT
DR. VICTOR MELTZER	DR. WILLIAM J. PULLEY
DR. ALFRED MEYER	DR. ED. QUINTARD
DR. WILLIAM H. MEYER	DR. FRANCIS M. RACKAMANN
DR. WILLY MEYER	DR. MILTON RAISBECK
DR. MICHAEL MICHAILOVSKY	DR. R. G. REESE
DR. G. N. MILLER	DR. FREDERICK W. RICE
DR. JAMES A. MILLER	DR. JOHN H. RICHARDS
DR. JOHN J. MOOREHEAD	DR. A. F. RIGGS
DR. A. V. MOSCHOWITZ	DR. HENRY ALSOP RILEY
DR. ELI MOSCHOWITZ	DR. BERNARD L. ROBINS
DR. ABRAHAM MOSS	DR. J. C. ROPER
DR. ARCHIBALD MURRAY	DR. BERNARD SACHS
DR. JOHN P. MUNN	DR. FORDYCE B. ST. JOHN
DR. WALDEN E. MUNS	DR. WILLIAM P. ST. LAWRENCE
DR. P. W. NATHAN	DR. T. W. SALMON
DR. A. E. NEERGAARD	DR. BERNARD SAMUELS
DR. HAROLD NEUHOF	DR. B. J. SANGER
DR. WALTER L. NILES	DR. REGINALD H. SAYRE
DR. VAN HORNE NORRIE	DR. OSCAR M. SCHLOSS
DR. N. R. NORTON	DR. HERBERT W. SCHMITZ
DR. W. P. NORTHRUP	DR. H. J. SCHWARTZ
DR. ALFRED T. OSGOOD	DR. L. L. SHAPIRO
DR. H. McM. PAINTER	DR. HOWARD F. SHATTUCK
DR. ELEANOR PARRY	DR. E. P. SHELBY
DR. W. B. PARSONS	DR. WILLIAM H. SHELDON
DR. STEWART PATON	DR. H. M. SILVER
DR. HENRY S. PATTERSON	DR. ALAN DEFOREST SMITH
DR. RICHARD M. PEARCE	DR. MARTIN DEFOREST SMITH
DR. MARSHALL C. PEASE, JR.	DR. THAYER A. SMITH
DR. CHARLES H. PECK	DR. R. GARFIELD SNYDER

ASSOCIATE MEMBERS—*Continued*

DR. F. P. SOLLEY
DR. F. E. SONDERN
DR. J. BENTLEY SQUIER
DR. NORBERT STADTMULLER
DR. ANTONIO STELLA
DR. ABRAM R. STERN
DR. A. R. STEVENS
DR. GEORGE D. STEWART
DR. LEO STIEGLITZ
DR. RALPH G. STILLMAN
DR. PHILIP STIMSON
DR. WILLIAM S. STONE
DR. A. McI. STRONG
DR. MILLS STURTEVANT
DR. A. S. TAYLOR
DR. A. M. THOMAS
DR. CORNELIUS J. TYSON
DR. F. T. VAN BEUREN
DR. PHILIP VAN INGEN
DR. ALBERT VANDER VEER

DR. H. N. VERMILYE
DR. S. WACHSMANN
DR. R. P. WADHAMS
DR. JOHN B. WALKER
DR. GEORGE G. WARD
DR. JAMES SEARS WATERMAN
DR. R. W. WEBSTER
DR. JOHN E. WEEKS
DR. WEBB W. WEEKS
DR. HERBERT WIENER
DR. RICHARD WIENER
DR. LINSLEY R. WILLIAMS
DR. A. O. WHIPPLE
DR. H. B. WILCOX
DR. MARGARET B. WILSON
DR. DAN H. WITT
DR. J. OGDEN WOODRUFF
DR. A. M. WRIGHT
DR. J. H. WYCKOFF
DR. CHARLES H. YOUNG
DR. JOHN VAN DORAN YOUNG

HONORARY MEMBERS

DR. JOHN J. ABEL
PROF. J. G. ADAMI
PROF. C. A. ALSBERG
PROF. J. F. ANDERSON
PROF. LEON ASHER
PROF. E. R. BALDWIN
PROF. JOSEPH BARCROFT
PROF. LEWELLYS F. BARKER
DR. WM. M. BAYLISS
PROF. F. G. BENEDICT
PROF. R. R. BENSLEY
DR. A. BIEDL
DR. WALTER R. BLOOR
DR. J. BORDET
PROF. WILLIAM T. BOVIE
PROF. A. CALMETTE
PROF. W. B. CANNON
PROF. A. J. CARLSON
PROF. W. E. CASTLE
PROF. CHARLES V. CHAPIN
PROF. R. H. CHITTENDEN
PROF. H. A. CHRISTIAN
PROF. E. G. CONKLIN
PROF. W. T. COUNCILMAN
DR. E. V. COWDRY
PROF. G. W. CRILE
PROF. HARVEY CUSHING
PROF. ARTHUR CUSHNY
PROF. H. H. DALE
PROF. HENRY H. DONALDSON
PROF. GEORGE DREYER
DR. LOUIS I. DUBLIN
PROF. DAVID L. EDSALL

PROF. JOSEPH ERLANGER
PROF. HERBERT M. EVANS
PROF. WM. FALTA
PROF. OTTO FOLIN
PROF. F. P. GAY
PROF. EVARTS A. GRAHAM
PROF. J. S. HALDANE
PROF. ROSS G. HARRISON
PROF. SVEN G. HEDIN
PROF. LUDWIG HEKTOEN
PROF. L. H. HENDERSON
PROF. YANDELL HENDERSON
DR. F. GOWLAND HOPKINS
PROF. W. H. HOWELL
PROF. JOHN HOWLAND
PROF. CARL G. HUBER
DR. WALTER A. JACOBS
PROF. JOSEPH JASTROW
PROF. H. S. JENNINGS
PROF. E. O. JORDAN
PROF. E. P. JOSLIN
DR. E. C. KENDALL
PROF. OTTO KESTNER
PROF. FRANZ KNOOP
PROF. ALBRECHT KOSSEL
DR. ALLEN K. KRAUSE
PROF. AUGUST KROGH
PROF. J. B. LEATHES
PROF. A. MAGNUS LEVY
PROF. PAUL A. LEWIS
PROF. THOMAS LEWIS
DR. C. C. LITTLE
PROF. A. S. LOEWENHART

HONORARY MEMBERS—*Continued*

PROF. A. B. McCALLUM
PROF. E. V. McCOLLUM
PROF. J. J. R. MacLEOD
PROF. F. B. MALLORY
PROF. W. McKIM MARRIOTT
PROF. L. B. MENDEL
PROF. HANS HORST MEYER
PROF. OTTO MEYERHOF
PROF. T. H. MORGAN
PROF. FRIEDRICH MULLER
SIR ARTHUR NEWSHOLME
PROF. KARL VON NOORDEN
PROF. FRED G. NOVY
PROF. G. H. F. NUTTALL
PROF. HENRY F. OSBORN
PROF. T. B. OSBORNE
DR. W. J. V. OSTERHOUT
PROF. G. H. PARKER
PROF. RAYMOND PEARL
PROF. CLEMENS PIRQUET
PROF. W. T. PORTER
PROF. T. W. RICHARDS
PROF. M. J. ROSENAU
PROF. MAX RUBNER
COL. F. F. RUSSELL
PROF. FLORENCE R. SABIN
SIR A. E. SCHAEFER

PROF. BÉLA SCHICK
PROF. PHILIP A. SHAFFER
PROF. HENRY C. SHERMAN
PROF. THEOBALD SMITH
PROF. E. H. STARLING
PROF. G. N. STEWART
PROF. C. W. STILES
PROF. C. R. STOCKARD
PROF. RICHARD P. STRONG
PROF. A. E. TAYLOR
PROF. W. S. THAYER
PROF. F. P. UNDERHILL
PROF. VICTOR C. VAUGHAN
PROF. MAX VERWORN
DR. CARL VOEGTLEIN
PROF. A. S. WARTHIN
PROF. J. CLARENCE WEBSTER
PROF. WILLIAM H. WELCH
PROF. H. GIDEON WELLS
PROF. KAREL F. WENCKEBACH
PROF. GEORGE H. WHIPPLE
COL. EUGENE R. WHITEMORE
PROF. E. B. WILSON
PROF. J. GORDON WILSON
PROF. S. B. WOLBACH
PROF. R. T. WOODYATT
SIR ALMROTH E. WRIGHT
DR. R. M. YERKES

DECEASED MEMBERS

DR. ISAAC ADLER	DR. ABRAHAM JACOBI
DR. SAMUEL ALEXANDER	DR. EDWARD G. JANEWAY
DR. W. B. ANDERTON	DR. H. H. JANEWAY
DR. PEARCE BAILEY	DR. THEODORE C. JANEWAY
DR. BOLTON BANGS	DR. E. L. KEYES
DR. CARL BECK	DR. FRANCIS P. KINNICUTT
DR. HERMANN M. BIGGS	DR. HERMANN KNAPP
DR. J. B. BORDEN	DR. GUSTAV LIANGMAN
DR. DAVID BOVAIRD	DR. EGBERT LE FEVRA
DR. S. M. BRICKNER	DR. CHAS. H. LEWIS
PROF. T. G. BRODIE	DR. JACQUES LOEB
DR. F. TILDEN BROWN	DR. SIGMUND LUSTGARTEN
DR. JOSEPH D. BRYANT	DR. CHAS. MCBURNEY
PROF. HANS CHIARI	DR. GEORGE MCNAUGHTON
DR. EDWIN B. CRAGIN	DR. W. B. MARPLE
DR. FLOYD M. CRANDALL	DR. S. J. MELTZER
DR. JOHN G. CURTIS	DR. CHARLES S. MINOT
DR. E. K. DUNHAM	DR. S. WEIR MITCHELL
DR. AUSTIN FLINT	DR. JAMES F. NAGLE
DR. JOHN A. FORDYCE	DR. SELIAN NEUHOF
DR. JOSEPH FRAENKEL	DR. GODFREY R. PISEK
DR. C. Z. GARSIDE	DR. G. R. POGUE
DR. EMIL GRUENING	DR. WILLIAM M. POLK
DR. WILLIAM S. HALSTEAD	DR. NATHANIEL B. POTTER
DR. H. J. HAMBURGER	DR. T. M. PRUDDEN
DR. FRANK HARTLEY	PROF. J. J. PUTNAM
DR. CHRISTIAN A. HERTER	DR. C. C. RANSOM
DR. PHILIP HANSON HISS	DR. ANDREW R. ROBINSON
DR. A. W. HOLLIS	DR. E. F. SAMPSON
DR. AUGUST HOCH	PROF. ADOLPH SCHMIDT
DR. EUGENE HODENPYL	PROF. W. T. SEDGWICK
DR. JOHN H. HUDDLESTON	DR. WILLIAM K. SIMPSON

DECEASED MEMBERS—*Continued*

DR. A. ALEXANDER SMITH
DR. RICHARD STEIN
DR. H. A. STEWART
DR. L. A. STIMSON
DR. OSCAR TEAGUE
DR. JOHN S. THACHER
DR. W. HANNA THOMPSON

DR. WISNER R. TOWNSEND
DR. R. VAN SANTVOORD
DR. H. F. WALKER
PROF. A. D. WALLER
DR. RICHARD WEIL
DR. JOSEPH E. WINTERS
DR. H. F. L. ZIEGEL

CONTENTS

	PAGE
"Organotherapy"	27
DR. A. BIEDL, Professor of Experimental Pathology, University of Prague, Czechoslovakia.	
"The Utilization of Fat in the Animal Body"	39
DR. WALTER R. BLOOR, Professor of Biological Chemistry, University of Rochester, N. Y. School of Medicine and Dentistry.	
"Chemotherapy of Protozoan and Bacterial Infections"	67
DR. WALTER A. JACOBS, Rockefeller Institute for Medical Research, New York.	
"Etiology and Prevention of Simple Goitre"	96
DR. DAVID MARINE, Director of Laboratories, Montefiore Hospital, New York.	
"Alterations of Intrapleural Pressure and their Significance"	123
DR. EVARTS A. GRAHAM, Professor of Surgery, Washington University School of Medicine, St. Louis, Missouri.	
"Physiological, Chemical and Clinical Studies on Pituitary Principles" ..	154
DR. JOHN J. ABEL, Professor of Pharmacology, Johns Hopkins Medical School, Baltimore, Md.	
"The Function of the Anterior Hypophysis"	212
DR. HERBERT M. EVANS, Professor of Anatomy, University of California, Berkeley, Cal.	
"Renal Secretion and Renal Disease"	236
DR. LUDWIG ASCHOFF, Professor of Pathology and Pathological Anatomy, University of Freiburg im Breisgau, Germany.	

ORGANOTHERAPY

ORGANOTHERAPY ¹

DR. ARTHUR BIEDL

Professor of Experimental Pathology, University of Prague, Czechoslovakia

WE mean by organotherapy the administration of animal tissues, juices, preparations and substances drawn from animal organs and tissues as medicine. Organotherapy comprehends a very large region, the limits of which may be extended by the imagination of an enterprising therapist as far as it is possible to obtain the desired substances. Theo- and omophagy rituals, originally observed by all people—were founded on mystical and religious conceptions and moral world-views. Their source was a deep superstition apparently not to be extirpated. Modern times have secured the necessary help in the magic art of quacks on the one hand and in the pharmaceutical laboratories on the other hand for the practical testing of the opinion already expressed by Plinius that the diseases of the most various organs may be treated and healed by the administration of the corresponding organs of animals.

It would certainly be worth while to consider from the point of view of a medical historian this fertile field of human error, for which the noble aim of succouring the needy might supply at once the explanation and the excuse.

We would today draw attention to a much more limited province conquered by scientific research within the last three decades, laboured since with untiring zeal and by the help of all methods of modern biology. We are now gathering in some of the fruits of this field for the alleviation of human suffering and for the diminution of human infirmities. Our organotherapy is based upon the doctrine of the internal secretion and finds its motivating power in the chemical regulation of the body functions by the endocrine glands. In the short time at our disposal it would hardly be possible to give even a bird's-eye view of organotherapy as a whole. We must restrain ourselves. We can do

¹Delivered October 13, 1923.

this, because nowadays the study of the endocrine glands has supplied us with a number of organ-preparations and substances which have definite actions like pharmaceutical drugs. Nearly every day we get notice of new medical effects from organo-preparations. I do not purpose to deal with this point today for the simple reason, that all these remedies belong to the province of pharmacotherapy. For the explanation of these effects only the principles of pharmacology need be considered. It may be very remarkable that in the animal body substances are formed with the same action as the medicamenta hortis. To study the physiological significance of these substances in the metabolism of the body is a most fascinating task, indeed. But for the practitioner it seems to be of little importance, whether the means of help is drawn from the vegetable or animal world, whether it is inorganic matter or obtained in the laboratory of an organic chemist. Finally he would ask only whether the substance applied would yield the desired effect and under what conditions and by what dosage. All this information he can obtain satisfactorily from pharmacology, in which organotherapeutics are no longer differentiated from the therapeutics of other drugs. All necessary knowledge about adrenalin or pituitrin, their therapeutic use and dosage is gained in the study of materia medica. By these remarks it will be understood that I am leaving off that part of organotherapy which deals with the organotropic effects of organ preparations. But I would bear in mind another point, the so-called ergotropic effects. If we consider the methods of obtaining and the quality of the organ-preparations, the fact, that most of them are protein-containing extracts of organs and finally, that some medicines chemically defined produce under certain conditions the same activity on men and animals as protein substances administered parenterally, the question might arise as to whether organotherapy is not possibly a special protein therapy. At present we hardly know anything for certain concerning the latter. We have only a general starting-point given by the explanation of Weichardt that the parenteral protein-administration produces an increased functioning of the different organs, a higher activity of the

protoplasm. It cannot be denied, that organ-preparations may sometimes act in the same ergotropic way. Even more: often organotropic and ergotropic actions are combined. The only effect which hormones can cause, is an increase in one or more functions, sometimes positively, sometimes negatively. The crux of the question lies not in the increased function, but in the manner of its origination. Protein-therapy induces protoplasmic activity for and through the total body. It results from a different way of reaction and the whole organism takes part in this phenomenon. In organotherapy something else happens. An example might elucidate this. Testicular liquid, the extract obtained from the male sex-gland, produces increased functional activity, recognized by the father of organotherapy, Brown Séquard, and proved on himself. Later this was tested by the exact experiments of Zoth and Pregl. One is at first tempted to see here an ergotropic action. But as it can be shown that the same hyperactivity may occur not only by the injection of this liquid into the body, but also in the surviving muscle, we cannot explain it by ergotropy. And for this reason: that we defined ergotropy as the higher activity of the protoplasm by means of the whole organism. In this somewhat obscure region it is particularly necessary not to deviate from a definition once fixed. The abuse of the term anaphylaxis should be a warning.

In our discussion of today we might leave ergotropic action out of consideration, because the part of organotherapy treated by me concerns chiefly administration by mouth. Ergotropic actions are only to be seen after administering substances parenterally. As my special theme I should like to choose the substitutive organotherapy. It was to be determined how the lack of organs or the defect of their function could be made up. The first step was the implantation of the lacking organ. The changes in the vital processes produced by experimental excision of a gland could be prevented or neutralized by the implantation of the gland elsewhere in the body. The organ has only changed its place and it seems quite evident that this was not organotherapy in the strict sense of the word. A further step led to the method of Brown Séquard according to which the

juice of the organ is introduced into the organism in the hope of replacing the lacking function. From this somewhat elementary basis developed hormonotherapy, *i.e.*, the administration of substances which have a replacing action or an irritating effect on the remaining parts of the organ. Here we met with a real substitution of the function.

Practical experience proves that the results of animal experimentation can be fully applied to man. Organotherapy is effective in many cases of disease where an incretory organ is lacking or in a state of decreased activity. Let us take the well known example of the thyroid. On man we use the administration of thyroid first in cases of congenital absence of the thyroid gland, so-called sporadic cretinism or thyroaplasia, secondly in cases of complete extirpation of the goitrous thyroid gland, happily no longer practised today, and finally in those cases where thyroid is not completely absent, but the symptoms indicate a limited activity of this organ. This occurs in the myxœdema both of adults and infants and also in endemic cretinism. In the latter we applied the thyroid preparations on the supposition that cretinism corresponds to a real athyreosis. Thyroid therapy was and is an effective method of fighting endemic cretinism although our supposition was not entirely proved. I need not mention that in our days the prevention of goiter and endemic cretinism has made considerable progress here and in our countries by the extensive use of small doses of iodine based especially on the careful studies of David Marine. I need not dwell further on the astonishing results of substitutive therapy. It suffices to call to mind that a child with congenital lack of thyroid becomes completely changed both somatically and psychically after a few months of treatment. However surprising and gratifying the curative effect may be, the fact must at once be emphasised that we can never obtain a complete substitution of the lacking organ. Contrary to the view of Kocher, that thyroid feeding restores a man after thyroidectomy as though the gland were in his own body I pointed out more than ten years ago that substitutive organotherapy would and could not be more than an approximate imitation of nature and in no way

perfect. We supply the body with substances, which in normal conditions are insensibly produced according to need, but we know practically nothing about the quantity normally produced and needed. Until a few years ago we could hardly measure the value of thyroid preparations, we could only estimate them by their effect. For that reason we constantly made mistakes in over- or underdosing. With Kendall's thyroxin, which seems to be the active principle, or, let us say, one of the active principles of the thyroid gland, we overcame at least partially these difficulties. But we should bear in mind the following: Even in those cases in which the thyroid therapy is destined to give the best results, such as thyroaplasia or myxœdema, we saw, and all who have had any experience of their own must agree, that the substitution fell short of complete restoration. We are at last forced to the more effective method of implantation. In order to prevent disappointment I should at once mention that this method is also limited. Even organs implanted with full success have but a temporary existence: this is a biological law. All transplants become in the body reconstructed and newly built and they are always in danger of perishing because of insufficient nutrition. At any rate they can remain but months or at most a few years with sufficient function.

If we recognize the limits of organotherapy we are saved from the disillusion of great expectations. If even in the simplest cases of deficiency of an endocrine organ such as the thyroid the substitution sometimes fails, we can hardly expect that in complicated cases, such as inflammations or malignant tumors of compound organ-systems such as the suprarenal bodies, consisting of two parts; the adrenal and interrenal system, the administration of gland-preparations would give satisfactory results. In failures of organotherapy we must face the question of the cause of this. An answer is given us by a closer knowledge of the mode of functioning in the endocrine system. Deficiency of one gland not only entails lack of action directed by it, but also a disturbance in the harmony of the whole system. By the absence of thyroid the body loses not only the thyroid hormones and is affected by the lack of these, but at the same time the regulation

of a number of organs connected with the thyroid is absent or limited. Or it would be better to say, that by the absence of the thyroid the whole endocrine system is disturbed. Briefly athyreosis means the loss, if not of the conductor, at least of the first violin of the incretory concert.

There is another consideration. All of us accept too easily the fact, that the pathological disturbance of a gland results in the diminution of its activity in every direction. But this is undoubtedly not the case. The gland-influences, originally different in quantity and quality, will certainly be altered to a different degree. The practical consequence of these considerations must be that the better we know the sphere of influence of an individual organ and the more we can recognize the responsibility of the incretory organ for a given alteration or illness, the better the effects of substitution must be. Organotherapy may be insufficient in the case of total lack, but in a smaller though well defined deficiency of hormones, in all cases of so-called *petite insuffisance* we shall obtain the best results, if we have an exact knowledge of the functional activity of endocrines in general. It seems to me not necessary to point out in detail the examples in which the administration of thyroid has been beneficial to patients in cases of sleeplessness, of constipation, of obesity, of eczema or baldness. We see the successful results of organotherapy everywhere, only we must not expect too much.

In order to improve the medical instrument of organotherapy the main condition is the knowledge of the endocrine constitution in general and the real evaluation of the part played by the various compounds of the endocrine system. Clinical observation has taught us the whole picture of diseases of the single glands and has given us the means to estimate the endocrine significance of one symptom or of a number of them. On the other hand, we have been enabled to estimate the influence of one or more endocrine organs in disposition to a disease, or on the course and end of it. Now we know that the endocrines singly and in their totality have an effectual influence on birth, formation and development, on the somatic and psychic condition of the individual, that they determine his metabolism and the

activity of all his tissues, organs and systems. The endocrine formula of a person is his fate. It is manifested in his bodily constitution externally in his habit, functionally in the mode of reaction and in the quantity of work of his organs. Only the detailed examination of the bodily constitution and the somatic and psychic reactions will put us in the position to judge and value correctly the incertory components.

I should like to demonstrate by an example of the estimate of metabolism the worth of an exact hormonal judgment. Usually we deduce the metabolic equilibrium from the constancy of body-weight and we compare the magnitude of the needs in nourishment and energy with the standard values obtained from former studies. But it seems to be clear that actually different standard values must serve for normal function, for the overfunction and the underfunction of the individual endocrine glands. Hence the terminology hypo- and hyperthyroidism, hypo- and hyperpituitarism, hypo- and hypergenitalism are in reality only schematic, separating conditions which are not determinable quantitatively and which cannot be separated from each other by sharp boundaries. Therefore, we are compelled to prepare individual standard values. Average values, extremely important for statistics and political economy, have lost their significance in the clinic. In the individual we see far-reaching differences, not only in the amount but also in the kind of food ingested; we find differences in the nutritive conditions in which no parallelism can be found between the amount of food-intake and the body-weight which is used as an index of the condition of nutrition; in fact, very often a marked discrepancy exists. The same is confirmed by the results of metabolism-experiments. The values obtained from different persons, even when reckoned on the basis of body-weight or body-surface, vary within such wide boundaries that one cannot use the average values obtained in the fasting-condition for the calculations. Let us pass along. The basal metabolism as a measure for the work, which in a resting, fasting organism is done by the vegetative organs and for the maintenance of the normal muscle-tone, must change with a change in the operative factors. The endocrine system

stands in the first rank as a leader of these phenomena. In a similar manner the influence of the incretory glands on the efficiency of production—the German: *Leistungszuwachs*—is also important in that, appetite, nutrition and impulses to movements are dependent upon them in part, as are the strength and duration of muscular activity. Now the small immeasurable movements of daily life whose number and magnitude are dependent upon the agility and the temperament of the individual, or more accurately upon his endocrine glands, yield in their totality undoubtedly an energy-consumption that is not to be discounted. That production-efficiency differs in different ages, according to the magnitude of the muscular activity is self-evident. That metabolism differs in different ages is a matter of record. But little attention has been paid to the fact that primary differential endogenous factors are determinative for the differences in muscle-tonus and for the different stimuli to muscular activity commonly shown by the impetuosity of youth, the restraint of the movements of the adult, and by the increased comfort-seeking of the middle-aged and the high degree of rest needed by the aged.

However obvious my point of view may appear, or however little it contains of what is new or surprising, it is a matter of great interest that when individual cases are subjected to my method of consideration and investigation, we thereupon acquire definite directions for an efficient therapy. We turn to the exact method of experiment. After a careful weighing of the data given by the clinical signs and after the establishment of the individual standard by metabolism-experiments we administer active amounts of one or the other or several glands of internal secretion and determine whether or not a change in metabolism can readily be obtained. A constant detectable marked increase in metabolism which is caused by a certain hormone or a definite hormone-combination and by this only proves indeed that the individual in question has a lower exchange as a personal standard than is stipulated in hormone-equilibrium. At the same time we have also ascertained the cause of the equilibrium-disturbance.

Practically this method of research shapes itself up rather simply; its results are none the less striking. We use the body weight as a measure of metabolism. In the short time at my disposal I am sorry not to be able to give further details, but I could cite many cases from my experience in which, on the basis of the clinically determined endocrine formula in hormone research, the disturbing glands were recognized as it were at the first attempt at hormone-investigation, and the further study of other hormones, by their negative, results confirmed the exclusive significance of the single organ. Many cases of our own taught us the correlation between metabolism and the whole body organization with a dependence on the endocrine apparatus. They have shown us also the prevalence of one link of the endocrine chain which occurs in many cases, which however is not always schematically demonstrated and must first be proven by experimental tests. One should be particularly warned against the tendency of modern times "to cure all from one one point."

I dare to hope you will recognize my right at this time to say some words concerning the question of averting senescence or rather rejuvenation a subject from the time of Brown Séquard of great dramatic interest. The "father of the internal secretions" placed the gonads as the axis of the problem of old age, and it is perhaps not superfluous to call to mind that the communications of this old man on his studies with the *liquide testiculaire*, which he consistently administered to himself, had such scintillating confirmatory results. Similarly the evident attention which was directed to him for thirty years still survives with us today. But if today one should make the gonads answerable for senescence and if one should abolish completely or avert the whole complex of old age by the reanimation of one type of cells, then one would overlook the important development of the field of internal secretion, the recognition of the significance and the effect of the other endocrine glands and of their coöperation. Just as all bodily conditions and their chemical reactions are under the influence of the entire hormone apparatus, so is old age. This condition is regulated and directed in the degree

and course of its development, not only by the testicles or ovaries, particularly not by the interstitial cells, but just as well by the thyroid and thymus glands, by the pituitary and the pineal, by the interrenal and adrenal system, and perhaps by many other organs and systems, in short by the whole endocrine apparatus, by its single parts, but before all by the harmonious coöperation of these parts. In premature old age all endocrine glands can be considered at the pathogenetic centres. Rejuvenation effects can doubtless be obtained by every organotherapy suitable to the disturbed endocrine formula. Nobody could object to considering the curative effects of the thyroid in cretinism or the anterior pituitary therapy of ateleiosis as real rejuvenation. I will not be prolix. Here I can only touch on the fact that old age and youth are questions of the endocrine harmony.

Endocrines are not only glands of metabolism, but the real glands of growth. Since the experiments of Gudernatsch on tadpoles we have learned a great deal about the influence of different endocrine glands on the growth, development and metamorphosis of lower animals. Experiments on man and other mammals give us sufficient evidence for the supposition, that here too growth is determined by the endocrine system together with other factors. Remembering the processes of overgrowth in acromegaly and gigantism, surely in connection with and in dependence upon the pituitary we may expect in this organ, in the anterior lobe a hormone of growth.

On the basis of this consideration I recommended the trial of the treatment of certain cases of dwarfism by anterior pituitary some ten years ago. At that time the animal experiments in this line were not very encouraging. However I could quote a considerable number of cases in which this method had given an astonishing stimulus to growth. It was very remarkable that all these cases of success were with persons at the beginning of puberty. The increased growth was always connected with a hastening of the sex-development. Cases of inhibited growth of younger years, such as between 8 and 13, hardly reacted at all, even if all the other signs pointed to a hypophyseal origin. With younger children the stimulation to growth was again more or

less marked. In the works of Brailsford Robertson I found the experimental basis for the explanation of these observations. He could demonstrate on mice the existence of three normal periods of growth and proved that a lipoid substance obtained from the anterior pituitary and called by him tetelin, considerably hastens growth in the third period, while it has no influence in the second period, though it certainly effects an increase in body-weight. He concludes that a change of growth is only possible in the sensitive period. We have known for a long time that several periods of growth exist in man. The first stretch from 5 to 7, and a second during the time preceding puberty. We should really consider three periods of growth with men as with mice, because there is no doubt that the curve of growth in the first year of life is a peculiar and especially characteristic one.

My own experiments in feeding hypophyseal substance show a negative result during the insensitive period between 7 and 13, a pregnant effect during the second stretch of puberty and a noticeable effect during the first stretch. It appears to me important to emphasize that all trials with growth-affecting substances should be undertaken during the sensitive periods.

In agreement with this is the experience which I could gain by the application of hypophyseal substance in another direction. Fliess was the first to apply this medicine for enuresis in children. I was able to effect a complete cure on a six-year-old child and on two cases in boys of 14 and 15 years. In all other cases, on children between 6 and 13 and especially on grown-up men, soldiers suffering during the war from enuresis the pituitary treatment was absolutely without effect. It seems to me that here also the sensitive period is in play.

In the limited time at my disposal I must be content to quote only these few examples from the extended field of organotherapy. In order not to disappoint too optimistic hopes I have only mentioned that which has already been proved and is perhaps already known. I believe I could state some other encouraging facts too. The organotherapy I have spoken of holds good only on the condition that we can apply preparations of the best quality

and in unlimited quantities. Physicians must insist on the pressing claim that all organ-preparations have to be examined in regard to their effect by all physiological methods. A number of tests are at our disposal and we should not tire in our search for new methods. A very grateful task indeed! All I have said of course takes for granted the application of proved and standardised preparations.

I should like to summarise. Organotherapy is no *elixir* for every case. It includes a number of effective and well studied agents obtained from the animal body. Organotherapy as substitution is also not a sovereign method against all diseases of the endocrine glands. We have acquired the means of relieving symptoms resulting from an unsatisfactory functioning of the endocrine system. This is not very much, but enough to stimulate to fresh efforts.

THE UTILIZATION OF FAT IN THE ANIMAL BODY¹

DR. W. R. BLOOR

Professor of Biological Chemistry, University of Rochester, N. Y., School of Medicine and Dentistry

ATTENTION has recently been sharply focussed on fat and its intermediary metabolism because of the controversy regarding the use of high fat diets in diabetes. It would be premature to enter into the merits of the discussion at the present time, since the subject is in the stage of rapid development, with new facts and conceptions appearing with each new publication. But the differences of opinion and the resulting experimentation are of the greatest importance in the clarification of our ideas on intermediary fat metabolism and it has seemed to be therefore a particularly good time to review our knowledge and determine just where we stand in this interesting but difficult field.

The place of fat among the foodstuffs.—Ordinarily, fat supplies about one-fifth of the caloric requirement and the percentage is increased with increased energy requirement and in diabetes, owing to the failure to burn carbohydrates. Fat is the most concentrated foodstuff and the only one stored in large amounts. It is not water soluble and probably for this reason is the most difficultly digestible of the foodstuffs. Burned alone or in large proportion, it is not an economical fuel, Krogh and Lindhard,¹ having shown that the waste of energy in work done on fat as compared with carbohydrates was about 10 per cent., and in addition that the fatigue induced by the work was considerable and often excessive with fat as compared with carbohydrate (acidosis excluded). When fat supplies over 80 per cent. of the energy requirement, the factor of incomplete combustion with production of acetone body acids becomes a possible source of danger.

¹ Delivered November 3, 1923.

Chemical Nature and Relations.—The fats are compounds of the higher fatty acids with glycerol, but for purposes of this study they may be considered simply as fatty acids, the glycerol component playing a relatively insignificant part. The fatty acids ordinarily found in food fats are sixteen and eighteen carbon-atom straight-chain compounds, none of them highly unsaturated. An exception is the important fat, butter, in which there is a notable proportion of the short-chain fatty acids. In the consideration of the utilization of fats it is becoming increasingly necessary to take into account various other compounds of fatty acids in the organism, substances which are ordinarily classified as lipoids, among which are the various compounds of which lecithin is a type—compounds of the fats with phosphoric acid. The part taken by phosphoric acid in the metabolism of the carbohydrates is already well recognized. The importance of phosphoric acid combined as lecithin in the utilization of the fatty acids is supported by a good deal of data, to which reference will be made later. The complex aromatic alcohol cholesterol appears also to be closely connected with the metabolism of the fatty acids through its fatty acid esters. Lipoids of other types—compounds of fatty acids with carbohydrate radicals, etc., are largely confined to the brain and nerve tissue and their presence there gives a basis for some interesting speculation because of the close relationship found to exist between the fatty acids and the carbohydrates in metabolism.

The fact that these various substances are compounds of fatty acids and undoubtedly concerned in their metabolism indicates that a separate classification into fats and lipoids is indefensible and that both should be grouped together as phases of their common metabolism. A classification embodying these ideas and with the fatty acid as the basis of classification was published a year or two ago.²

It appears possible also to divide the fatty acids of warm blooded animals into two functional groups, according to whether or not they are in immediate use in the body, as active and inactive fatty acids. The division for warm blooded animals

may somewhat arbitrarily be made as follows: those acids more saturated than oleic acid are classed as active, all others, including oleic and the saturated acids, as inactive. The main reason for this division is that it is rare to find in the stored and therefore inactive fat of warm blooded animals any acid less saturated than oleic, while the fatty acids of the tissues which are continuously active, such as heart, kidney and brain, are largely of the more highly unsaturated type. The classification does not apply to the fatty acids of cold blooded animals nor of plants, for the fatty acids found in the fat stores of these organisms are often highly unsaturated. The reason for the difference appears to be that a stored fat in any organism has to satisfy two requirements (a) it must be stored in such a form as to be available, *i.e.*, liquid, at any temperature to which the living organism may be submitted, which means that in cold blooded animals and plants there must be a considerable proportion of the highly unsaturated (low-melting) fats present and (b) the stored fat must not be spontaneously oxidizable at the environmental temperatures, which necessarily limits the amount of the highly unsaturated fat present. In warm blooded animals, where the bodily temperature is nearly constant at a relatively high temperature, a suitable admixture of olein with the saturated fats is sufficient to preserve fluidity.

The Metabolism of the Fats.—Certain phases of the subject have already been dealt with in lectures before the Society—the function of the liver in fat metabolism, by Leathes, the rôle of fat in diabetes by Allen and the balance between the carbohydrates and fat in fat combustion by Shaffer. For that reason most parts of the subjects covered by them may be passed over rather briefly. Also, only a brief treatment will be given to the processes of digestion, absorption and transport, since very little that is new can be said about them and since it seemed desirable to spend most of the time in a discussion of the intermediary metabolism of the fatty acids. Furthermore, since the time available is short, it seems wise to pass over with a brief reference, certain phases of the subject which in a dis-

cussion of this kind might well be given major mention but of which excellent reviews are available.

The Digestion and Absorption of the Fats.—The peculiarities in the behavior of fats in digestion and absorption may be referred largely to the fact that they are insoluble in water. Because of this insolubility the changes in the stomach are ordinarily slight, although a lipase is present. Only when fat is present in an emulsified form (as, for example, milk) is there any notable amount of fat digestion in the stomach. Their insolubility may explain the fact that they inhibit, or perhaps rather do not stimulate, gastric activity and consequently are slow in leaving the stomach. So far as is known no absorption takes place in the stomach.

In the intestines—fats being insoluble in water, while the lipases are soluble, digestive action takes place only at the fat-water interfaces and is therefore effective only when the surface is greatly increased, as by emulsification. The main emulsifying agents are the soaps—the water soluble form of the fatty acids, but only second in importance is the bile, which appears to have been provided especially for the digestion of the fats, since it not only aids in forming and preserving the emulsion but by means of its salts dissolves large amounts of the fatty acids and is a very important factor in their passage through the intestinal wall. In the absence of bile very little fat absorption takes place. The form of absorption is uncertain, but presumably is as soaps or fatty acids, for the facilities for splitting the fats are so adequate that only traces of unsplit fat are found in the feces. If so, there is immediate synthesis in the intestinal wall (a marked difference from the other foodstuffs) for the absorbed material leaves the intestine as fat. The entrance into the blood stream of the absorbed fat takes place apparently only by way of the lymphatic system and it is thus not submitted, like the other foodstuffs, to the censorship of the liver, useless or harmful substances apparently being stopped at the port of entry in the intestine. Thus, the fats are transferred to the tissues from the food bodily, and not furnished to the cells in the form of building stones, as in the case with the other foodstuffs.

The region of absorption of the fats in the intestine is apparently more limited than that of the other foods. There is no absorption from the large intestine and, in the small intestine, fat, if not quickly absorbed, may escape altogether, as shown by the fact that large amounts of fatty acids may reach the large intestine and the feces, indicating a failure in absorption and not in digestion. A probable explanation of the failure of absorption may be found in the fact that the bile salts are absorbed from the intestine to be used over again (circulation of bile) and since these are known to be very important³ for fatty acid absorption, any fatty acids left unabsorbed after the absorption of the bile would be wasted.

The fats thus differ fundamentally from the other food-stuffs in their manner of digestion and passage from the intestine to the blood stream. All evidence points to their complete splitting into fatty acids and glycerol and their absorption as soaps, *i.e.*, their water soluble form, but the soaps, because of their large molecule and their ready hydrolysis and powers of aggregation, probably do not behave as crystalloids but rather as colloids with fairly large particles, so that the splitting of the fats probably does not greatly simplify their handling during absorption, hence the great importance of the solvent powers of the bile acids.

An entirely separate channel is provided for the conveyance of fats to the blood. They reach the circulation by way of the lymph vessels and apparently do not pass in any appreciable amounts into the intestinal blood system. The most recent efforts to demonstrate their increased presence there⁴ failed in finding any definite difference in the fat content of the jugular, portal or mesenteric veins. However, the methods used were not exact enough to show small differences which might be significant. Some older work by Joannovics and Pick⁵ has a bearing on this point, but only serves to complicate the situation. Their experiments were as follows: Dogs were fed with oil having an iodine number of 120-130 and their liver fat examined at the height of digestion. It was found that the fat of the liver had doubled in amount (as compared with normal animals) and had

an iodine number higher than the fat fed and much higher than the liver fat of animals on ordinary food—indicating an accumulation of fat in the liver during digestion and also a desaturation. In dogs with an Eck fistula there was no rise of liver fat nor increase of iodine number, indicating that normally the portal vein is an important factor in the accumulation of fat in the liver. As far as our present information shows, the fragments of the other foodstuffs produced by digestion reach the blood directly as such without synthesis in the intestinal walls. The fatty acids and glycerol are built up into fats again before they leave the intestinal walls, passing out from these cells across the lumen of the villus to the openings of the lymph system in a manner, the details of which are largely unknown. The reason for the synthesis and thereby the difference from other foodstuffs may probably be sought in the fact that soaps in the blood stream would be dangerous, due to their hydrolysis and consequent alkalinity and to the known hemolytic effect of some of them. The absorbed fat reaches the blood stream by the lymphatic system and mainly through the thoracic duct while none of the other foodstuffs enter by that way. In view of the similarity in fat content of the portal and general circulation, the fact that only about 60 per cent. of the absorbed fat can be recovered from the thoracic duct at its point of entry into the blood system was a considerable stumbling block until quite recently, when Lee⁶ was able to show that the thoracic duct was probably not the only opening by which the absorbed fat reached the blood stream.

The synthesis of fat during the passage through the intestinal cells gives an opportunity for certain adaptive changes. The possible changes may be (a) a rearrangement of the fatty acids attached to the glycerol molecule, producing mixed glycerides from simple ones or *vice versa*; (b) a saturation or desaturation of the fatty acids, with corresponding change of properties (c) a dilution with fat from nearby depots, especially the liver, (d) a selection at different stages of digestion of certain of the various fatty acids set free by digestion. Under suitable conditions

changes due to one or more of these causes have been shown to take place. For example, after feeding a high-melting saturated fatty acid such as stearic acid, the chyle contains considerable amounts of low-melting unsaturated fat⁷. And when moderate amounts of triolein—an unsaturated, low-melting fat—is fed, the fat of the chyle contains measurable amounts of saturated high-melting fat. The mechanism appears only moderately effective, for it is well known that when a large amount of fat is fed it is transferred to the fat depots without demonstrable change. The fact should be pointed out however that under normal conditions it is rare for fat enough to be taken with the diet to produce any definite effect on the fat of the depots, each animal storing a fat that is characteristic of the species and which probably represents a balance between utilization and storage of the food fat and of the fat synthesized from carbohydrate. The fat which reaches the blood, although originating in the food fat may thus, under suitable conditions, have quite different properties and the part taken by the intestinal wall in the modification of the fat passing through it is worthy of further investigation.

The fat enters the blood stream in the form of very finely divided particles variously called “fat dust,” hemakonia, chylomicrons, about $1\ \mu$ in diameter and with a pronounced Brownian movement. At the time when the fat reaches the blood, or soon after, there is to be noted an increase of lecithin (lipoid phosphorus) and sometimes cholesterol⁸ in the blood, the former of which is believed to have its origin in the fat and to be a stage in its utilization. The advantage of a change from fat to lecithin in the blood is easily seen. The blood lecithin is to all intents and purposes water soluble and the greater ease of handling such a substance in a watery system is apparent. The close chemical relationship of lecithin to fat makes the change relatively simple and the greater sensitiveness of lecithin to chemical change opens a path for the later series of metabolic changes. The advantage of an increase of cholesterol in the blood is not so apparent, since, although it forms esters with the fatty

acids, these are chemically more inert and if anything, less soluble than the fats.*

Nothing definite is known regarding the transference of the blood fat to the tissues, but the indications are that there is more than one way by which it is accomplished. During the time of high fat content of the blood, fat is found collected at various points of the endothelial lining of the blood vessels and it is probable that certain of the cells have the power of engulfing fat particles that come within their reach, with the probability that they can pass them on into the tissues. Attention should be called at this point to the ability possessed by many living cells to reduce fat to a form so finely divided as to be invisible in the microscope, therefore, presumably colloidal. Also, there can be no question but that the transformation of fat into the water soluble lecithin is of great importance in its passage from the blood, as Meigs and his co-workers have shown for milk fat secretion.¹⁰

Nor can anything definite be said of the passage of fat out from the tissue cells into the blood when needed for combustion, except that it is probably not a simple process. Under most conditions the discharge from the fat tissues into the blood is not rapid enough to produce any marked changes in the lipoidal composition of the plasma, but when the storage cells are filled to capacity the stimulus to empty may cause an excessive discharge sufficient to produce not only an increase in lecithin and cholesterol but also a visible lipemia. This excessive discharge appears to depend on local instability of the fat stores, since the general fatness or leanness of the animal is apparently not an important factor.¹¹ When not disturbed by excessive inflow of fat the blood lipoids of both corpuseles and plasma bear a constant relation to each other. According to our present information,

* From the clinical point of view it is worth while noting that there appears to be a parallelism between the fat content of the blood as determined by a count of the visible particles⁹ and the content determined by chemical methods, indicating that the method of determination by count may have considerable clinical value in the study of fat absorption in the intestine.

there is little if any true fat (glycerides) to be found under these conditions in either plasma or corpuscles, cholesterol with its fatty acid esters (in the corpuscles, cholesterol alone) and lecithin (phospholipiod) making up the lipid content. All available data indicate that there is a constant balance between cholesterol and its esters on the one hand and cholesterol and lecithin on the other. The fact of a similar balance in most tissues has been established by the French workers in this field.¹² The balance between the lipoidal constituents of blood is closely maintained unless the increase in lipoids is very great, when there may be a disproportion. Thus, when there is a large and sudden increase of lipoids, as sometimes happens in diabetic lipemia and in the hemorrhagic lipemia in rabbits, the first constituent to increase is the fat, next the lecithin and latest the cholesterol, which generally continues to increase after the increases in lecithin have ceased, exhibiting thus the same sequence as is found during alimentary lipemia.¹³ After the lipemia is established the cholesterol and lecithin bear about the same relation to each other as in fasting blood. Even in the severe lipemia of diabetes and in the hemorrhagic lipemia of rabbits with a large increase of total lipid, this balance is pretty well preserved,¹⁴ which is in accord with the general conception of the constancy of the composition of the blood. The blood corpuscles are apparently not concerned in any but the alimentary lipemia, since in the persistent lipemias their lipid constant is about the same as in fasting blood.

Intermediary Metabolism.—Regarding the intermediary metabolism of the fats our present conceptions are largely due to the work of Leathes who embodied his researches and generalizations in a lecture before this Society in 1909. Put briefly, his belief is that the fats intended for immediate use, whether from the food or from the fat depots, are carried to the liver where the fatty acids undergo the first stages in their breakdown, which consists in the introduction of double bonds at various points in the long chain. These points thereby become points of weakness which are first attacked by oxidation with the result that the long chain is broken, yielding a series of fragments which are then disposed

of separately. Leathes believed also 'that a transformation of the fat to lecithin may take place in the liver and that the desaturated, phosphorized fats were then distributed by the blood to the tissues for utilization. Since Leathes' lecture considerable material has accumulated which has a direct bearing on his hypothesis and it is desirable to consider the application of this new work to his ideas.

The work of Leathes and his fellow-workers regarding the fact of desaturation can, I think, be accepted without question. Also there is no doubt that the liver has marked power of desaturation and that it is the most important desaturating organ. Whether other organs are concerned in desaturation is uncertain, since some of them, such as the heart, kidney, brain, contain highly unsaturated fatty acids and during fat mobilization their fat content increases somewhat, although to nothing like the extent of the liver. If the liver is to be regarded as the sole or main place of desaturation from which the unsaturated fatty acids are carried by the blood to the tissues for use, then it is necessary to demonstrate the presence of highly unsaturated fatty acids in the blood, which it has been possible to do in recent work.¹⁵ The liquid fatty acids of the fasting blood plasma of various animals were found to have iodine numbers up to 160 and averaging in beef blood 147, dog 155, pig 153 and sheep 118, numbers which indicate the presence in considerable amounts of fatty acids with at least two double bonds and, as was shown by the behavior of the acid mixture, probably some with more than two.

The second part of Leathes' hypothesis, the conception that lecithin is formed from fat in the liver as a stage in the utilization of fat and is the carrier of the unsaturated fatty acids from the liver to the tissues where they are to be utilized is not quite so readily acceptable in all its details. The idea of lecithin as an intermediate stage in certain phases of fat utilization, as noted above, is attractive and highly plausible. In addition to the finding of Leathes and others that lecithin is found in the liver, heart, kidney, muscles, brain, etc., where fat metabolism is ordinarily active, it is found to increase in the blood and particularly in

the corpuscles during the absorption of fat and in certain disorders of fat metabolism, where it keeps pace with increases in other lipoids. The researches of Meigs¹⁰ and his co-workers make it probable that blood lecithin is the mother substance of milk fat. Its close chemical relationship to the fats adds further weight to the belief, since it may be regarded as a phosphorized fat—a glyceride in which one of the fatty acids is replaced by a phosphoric acid residue. The resulting compound, in contrast to the fats, mixes readily with water to form a homogeneous mixture which, in the case of freshly prepared blood lecithin, is almost clear, forming thus a water soluble vehicle for the insoluble fats which must be of great significance in the utilization in a watery medium such as the animal body. In this connection also the fact that lecithin is an excellent emulsifier must be of great importance in preserving the solution in the blood of such insoluble substances as cholesterol and the cholesterol esters. Lecithin is much more active chemically than the fats. It is more readily hydrolyzed and more readily oxidized, so much so, indeed, that it is very difficult to prepare it in pure form for examination. Enzymes are present in blood and tissues which hydrolyze lecithin readily but attack the fats hardly at all.¹⁶ Our information regarding the tissue lecithins is becoming increasingly definite and accurate, due to the efforts of Levene¹⁷ and MacLean¹⁸ and it is found that the two fatty acids in combination in tissue lecithins are generally different and generally one saturated and one unsaturated. According to the most reliable work, the fatty acids ordinarily in combination in lecithin and the closely related cephalin are often of considerable degree of unsaturation, four double bonds being reported.*

The tissue lecithins are optically active, which fits in well

* In this connection attention is called to the significant fact reported by Levene¹⁹ that the highly unsaturated fatty acids of these lecithins and cephalins are split out by cobra venom, leaving a residue containing only saturated fatty acids (lyso-lecithins and lyso-cephalins) indicating that the unsaturated acid is more readily removed than the saturated acid. The relation of this finding to the transport of the unsaturated fatty acids by lecithin is evident.

with the belief that optical activity is closely related to the phenomena of life. Another property of the lecithins, which increases their importance in the consideration of conditions in the living cell, is their power of forming loose combinations with all sorts of substances, sugars, proteins and salts, a property which may well be valuable in preserving the unity of the protoplasm of living tissue. The lecithins are thus well suited for the part which they are believed to play in the transport and the initial steps in the intermediary metabolism of the fatty acids. By the substitution of a phosphoric acid residue for one fatty acid the fats become at once reactive and water soluble, ready to participate in all the changes of a watery medium. The idea that the desaturated fatty acids are formed into lecithin in the liver and as such are transported by the blood to the tissues is based on the fact that lecithins containing highly unsaturated fatty acids are to be found in the liver and also in the heart, kidney, and other organs and tissues. If they are transported in the blood, lecithins containing highly unsaturated fatty acids should be demonstrable there. Investigations on this point now in progress in our laboratory do not give an entirely satisfactory answer although they do not exclude the possibility. Iodine numbers of the fatty acids of the lecithin fraction of plasma lipoids are found to run generally between 80 and 90, with a few as low as 40 and an occasional one over 100. The fatty acids from the lecithin fraction are a mixture containing both solid and liquid acids. The exact amounts of each have not been determined, nor is it possible by the method used to extract the lecithin without partially destroying it. The results so far indicate that some of the blood lecithins are of the same type as the liver lecithin recently described by Levene^{17a} containing one saturated and one unsaturated fatty acid with an iodine number of from 160 to 180 and containing therefore probably two double bonds. The data are made more difficult of interpretation by the fact that in blood in the fasting condition, transport of saturated fatty acids from the fat depots to the liver must be going on simultaneously with transport of the unsaturated fatty acids from the liver to the active tissues but the

findings so far are, however, not incompatible with Leathes' hypothesis regarding unsaturated acid transport.

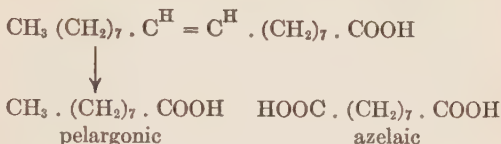
In connection with the mode of transport of the unsaturated fatty acids in blood, mention should be made of work now under way which has an important bearing on the function of cholesterol in fatty acid metabolism which brings us again to the deadwall of our ignorance as to the significance of this mysterious substance. Cholesterol is universal in its occurrence in tissues wherever there are other lipoids, sometimes free and sometimes in the form of esters of the fatty acids. It is a relatively stable substance and there is no satisfactory evidence that it is burned in the body.

It originates mainly in similar substances in the foods and like the sodium chloride of the blood there is ordinarily a constant flow of it through the organism, in with the food and out in the feces probably by way of the bile. In the blood, when not disturbed by food absorption, it bears a constant relation to lecithin and, in the plasma, to its own esters. In the corpuscles it is always found free. In the blood plasma it increases as the other lipoids increase and any increase of cholesterol due to overfeeding is followed by an increase in the other lipoids.²⁰ It is an insoluble substance and yet can be carried in apparent solution in the blood in amounts up to nearly two per cent., as in severe diabetes. It, or one of its slightly changed forms, is a main constituent of the fatty material of the skin secretions and finds its usefulness there largely because of its great stability under conditions where oxidation would be very easy—thin layers and exposure to light and warmth. When fed in relatively large amounts there is some evidence²¹ that it or its esters may deposit in the tissues and in the walls of the blood vessels. Its tendency to deposit in the gall bladder is too well known to call for comment and some writers have found a relation between high blood cholesterol and cholelithiasis.²² In blood plasma it occurs both free and combined in the proportion of about one part free to two parts combined. Based on the work of Hürthle,²³ the cholesterol esters of blood plasma have been believed to be the palmitate and oleate. Without comment on the presence

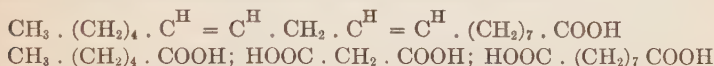
of palmitate, which seems well established, present work in this laboratory indicates that instead of cholesterol oleate the other ester is mainly linolate, or at least the ester of a fatty acid containing more than one double bond. There can be no reflection on Hürthle's work, which was accurately done, but the elementary analysis on which he depended for his information was not sensitive enough to distinguish between a compound with one and one with two double bonds and he did not make use of iodine number determinations to give information on this point. By fractionation of the lecithin-free blood lipoids with ethyl or methyl alcohol the least soluble and largest fraction is found to consist almost entirely of a compound which on analysis gives one molecule of cholesterol to one molecule of a fatty acid, with an iodine number varying from 120 to 170 and averaging about 145 (while for oleic acid the I.N. would be 90). The important fact is thus brought about that the highly unsaturated fatty acids found in blood plasma are carried to a considerable extent in combination with cholesterol. The significance of this fact is not altogether clear, for, while it seems to connect cholesterol closely with the intermediary metabolism of the fatty acids, the cholesterol esters are, in contrast with lecithin, very stable compounds. It brings to mind the familiar experiments of Knoop²⁴ who found on feeding phenyl derivatives of the fatty acids that the fatty acid chains were reduced two carbons at a time back to within one or two carbon atoms of the ring. It may be that cholesterol forms such an unburnable "handle" from which the fatty acids are burned.*

* Cholesterol and lecithin are known to be opposite in their effects on hemolysis, lecithin producing hemolysis under the influence of cobra venom (see note above) while cholesterol protects against hemolysis by saponin²⁵, agaricine and tetanolyisin²⁶. The occurrence of cholesterol esters of the highly unsaturated acids in blood and the fact that these acids appear to be readily split off from lecithin suggests a possible origin of the cholesterol esters of the unsaturated acids. When set free from the lecithin by esterases in the blood, which are known to attack lecithin, the acids are taken up by cholesterol, the combination serving two purposes—a neutralization of the hemolytic action of the unsaturated acids and the use of cholesterol in the further metabolism of the fatty acids, possibly as an unburnable residue from which the fatty acids are consumed.

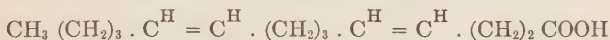
The next step in Leathes' hypothesis is the breaking by oxidation of the long chains at the points of desaturation in shorter chains the length of which depends on the number of double bonds. Thus ordinary oleic acid would yield azelaic and pelargonic acids.



The constitution of the linolic acids (two double bonds) is not well understood but such a one as below with one double bond between the 6th and 7th and one between the 9th and 10th would yield caproic, malonic and azelaic acids.



Fatty acids with two double bonds in other positions would yield different split products. Fatty acids with three double bonds are found in various organs—such a one as for example;



would yield valeric, glutaric (or butyric), succinic, or propionic. Arachidonic acid $\text{C}_{20}\text{H}_{32}\text{O}_2$, found by Hartley²⁷ in liver and by Levene^{17d} in liver lecithin, having four double bonds might be expected to yield mainly acids with less than five carbon atoms. (The position of the double bonds is a matter of not inconsiderable importance since it was pointed out by Lewkowitsch²⁸ that if the double bond is near the carboxyl group iodine absorption takes place very imperfectly and its extent varies with the iodine solution used.)

How far is desaturation carried? Fatty acids with more than four double bonds are suspected but not actually found, for with the increase of double bonds the susceptibility to oxidation increases so greatly that the practical difficulties render preparation very difficult if not impossible. However, it can readily be seen from the above that if desaturation with subsequent breaking went far enough any other method of oxidation, as for example β oxidation, would be unnecessary.

As noted above, desaturation as a preliminary stage in oxidation may be accepted as a fact. What evidence is there of the breaking of the chain into shorter fragments with separate oxidation as further required by the hypothesis? Before proceeding to a discussion of this phase of the subject it will be well to review briefly the present ideas regarding the method of final oxidation of the fatty acids. It is now pretty generally accepted that oxidation is brought about by the removal of the carbon atoms, two at a time, with a preliminary oxidation of the β carbon atom, which after the break becomes the terminal carboxyl group. The two carbon fragment produced by this method of oxidation is ordinarily assumed to be acetic acid. It should be noted that while the fatty acids of longer chain than acetic acid were found by Schotten²⁰ to be completely burned, acetic acid in the same amounts was only 90 per cent. burned, although smaller amounts were completely utilized. It is also interesting to quote in this connection the work of Loeb³⁰ and Friedman³¹ that acetates give rise to diacetic acid in perfused livers which are poor in glycogen. When much glycogen is present in the liver or when salts of propionic or valeric acids are present the change is entirely inhibited.³² It is not necessary at this time to go into the details of evidence regarding the β oxidation of the fatty acids, since this material is excellently reviewed by Dakin.³³ The outstanding facts are as follows: (a) the occurrence of the "acetone bodies" (β oxidized products) in the urine under certain conditions which may be summed up briefly as the lack of "available" carbohydrates in metabolism. These acetone bodies are now believed to originate mainly in the fatty acids and to represent their unburned residues. (b) The experiments of Knoop²⁴ feeding phenyl derivatives of the fatty acids and studying the excreted products gave strong indication that β oxidation is the prevailing method of oxidation in the animal body, both for chains with odd numbered carbon atoms as well as for those with even numbered carbon atoms, for saturated as well as unsaturated acids. The objection that β oxidation is not the rule *in vitro* has been largely overcome by the experiments of Dakin³⁴ with hydrogen peroxide at about body tempera-

ture, by which he showed that oxidation is the rule under these conditions which closely parallel those in the living body. Further evidence has been obtained by perfusion of surviving organs with various fatty acids (Embden³⁵), and by excessive feeding with fatty acids (Blum³⁶). β oxidation is thus shown to be very probable and in fact the only method of oxidation in the living body for which there is satisfactory evidence.

Incidentally it has been shown by these experiments that it is only the even numbered carbon atom fatty acids that yield acetone bodies. Odd numbered fatty acids do not—in fact Ringer³⁷ has demonstrated that the odd numbered fatty acids yield glucose to the extent of three carbon atoms to each chain, the sequence of changes being probably propionic to pyruvic to lactic acids and thence to glucose, α oxidation taking place when β oxidation is no longer possible, a probability given sound support by the recent work of Blum and Woring.³⁸

Many years ago it was shown by Magnus-Levy³⁹ that in the completely diabetic organism each fatty acid chain yielded only 1 molecule of β oxybutyric acid (the maximum yield of oxybutyric from 100 gm. of fat being 36 gm., *i.e.*, 3 mols. from each molecule of fat). His results of course refer only to even numbered chains which are the only ones occurring in natural fats. Work with shorter chain, even numbered fatty acids as noted above confirmed his findings for the shorter chains and there is at present no evidence to show that an even numbered fatty acid chain of whatever length can yield more than one molecule of acetone bodies. The bearing of these facts on Leathes' hypothesis of the breaking of the long chain acids at the double bonds into shorter chains with individual oxidation will be clear from the following. The shorter chains formed by breaking the long chain would yield either odd or even numbers of carbon atoms. The even numbered ones would each produce one molecule of acetone bodies, so that there would be as many molecules of acetone bodies as there were even numbered fragments formed. The odd numbered chains would yield glucose to the extent of three carbon atoms to each chain so that each pair of odd numbered fragments would produce one

molecule of glucose. However, the evidence is contrary to the production of sugar from fat, either in the natural diabetic or in experimentally produced diabetes. For example, Lusk has shown that fat feeding did not affect glycosuria either in phlorizinized dogs⁴⁰ or in diabetes⁴¹. Also⁴² that work which doubled fat catabolism did not alter the output of sugar in a phlorizinized dog. Some indirect evidence from respiration experiments is available, for example, the occasional R. Q. below 0.7 in diabetes and in depancreatized dogs, which is interpreted by some writers as evidence of change of fat to sugar, but is probably better explained by the desaturation of the fatty acids (see below). In the normal the most significant recent work on this point is that of Krogh and Lindhard¹ who as the result of studies of the Respiratory Quotient at various levels come to the conclusion that at quotients above 0.9 carbohydrate is transformed into fat and at quotients below 0.8 fat is transformed into carbohydrate. Without comment on the transformation of carbohydrate to fat which is known to take place readily, their conclusions with regard to the transformation of fat to carbohydrate may without injury to their conceptions be restated as follows: the abnormally low R. Q. is due to transformation of the fatty acid into substances containing *more oxygen* or *less hydrogen* than the fatty acid (but not necessarily carbohydrate). Such substances would be the desaturated or hydroxy fatty acids and so their findings may be brought into agreement with the desaturation hypothesis, since there would in these cases be utilization of oxygen without a corresponding output of CO₂. It is known that it is at just such times (low carbohydrate, high fat combustion) that there is a flow of fat to the liver as the result of its low glycogen content and the adherents of the carbohydrate from fat hypothesis have accepted this along with the reduced R. Q. as evidence for their theory.⁴³ It is interesting to note that Geelmuyden,⁴⁴ who has been one of the most insistent supporters of the fat to sugar hypothesis, accepts desaturation as the first stage in the transformation and gives the following diagram to illustrate what

he believes to be the first stage in the transformation of fatty acid to sugar.



The use of oxygen in this way and also possibly in the formation of hydroxy or ketone acids would explain the lowered quotient if the unsaturated acids were stored and Leathes has shown that they are stored to some extent in the liver. The apparent formation of fat from carbohydrate in these experiments can thus be shown to be really utilization of the fats and the apparent contradiction of Lusk's evidence regarding the transformation of fat to sugar is removed.

In summing up the evidence regarding the breaking of the fatty acids at the double bonds we are led to an absurdity. For, if more than one even numbered fragment is formed, then more than one molecule of acetone bodies per long chain fatty acid would be produced, which is not the case, while if odd numbered fragments are formed sugar would be produced from fat, which also appears not to be true. The conclusion is that the evidence is against the breaking of the long chains which then must therefore be reduced entirely by β oxidation. There are two possibilities of escape from this conclusion which should be considered. First, that sugar and acetone bodies may be produced in such amounts that they mutually consume each other, which is unlikely, although not beyond the bounds of possibility, and second, that the dicarboxy acids which may be formed by breaking the chain have a different path in metabolism from the monocarboxy acids. Evidence on the latter point is available from the work of Ringer,^{37b} who found that malic and succinic acids administered to phlorizinized animals yielded large amounts of glucose which, from his work on the odd numbered carbon fatty acids, he believed happened by loss of CO_2 , resulting in a three carbon acid. Some light is also thrown on the subject by the behavior of dicarboxy amino acids. Ringer and Lusk⁴⁴ found that aspartic acid yielded much extra glucose—by loss of CO_2

and oxidation at the α amino group. Similarly, glutamic acid went to glucose via succinic acid with loss of one carboxyl. In the case of these two amino acids, which differ by one carbon atom yet both yield glucose, the terminal carbon atom behaves differently, in one case disappearing, in the other persisting. Dakin⁴⁵ showed that arginine, ornithine and proline yield glucose presumably as Ringer suggests via succinic acid, while lysine, which would go to glutaric acid, apparently does not yield glucose. The work quoted shows as far as it goes that the dicarboxylic acids go to glucose and in that way are not different in their behavior from the odd numbered monocarboxylic acids. Of the other dicarboxylic acids not much is known. As regards glutaric acid, Ringer states that it yields neither glucose nor β oxybutyric acid. Leathes' own belief regarding the dicarboxylic acids was that they lose CO_2 and thus become monocarboxylic acids, after which are oxidized by β oxidation.

Taken altogether the available evidence gives no support to the assumption that the long chain fatty acids are broken into parts for oxidation but rather that the chain remains intact to the end, the only changes being the introduction of an unknown number of double bonds, which would render β oxidation easier.

A further possible change is the addition of hydroxyl groups at the double bonds. Hydroxy acids are found in the brain,⁴⁶ where they constitute about 25 per cent. of the solid acids. They have not been described as occurring elsewhere in animals, but, as they are formed readily from the unsaturated acids and occur in plants, it is probable that they would be found if looked for. The fact that they are relatively soluble in water offers another possibility in the consideration of the soluble forms of the fatty acids. Dakin's review of this phase of the subject leads him to the belief that the unsaturated fatty acids may take up water to form optically active saturated hydroxy acids which then undergo further oxidation.³³ The conditions respecting the fatty acid compounds of the brain are worthy of comment. The energy exchange of brain and nerve tissue is relatively minute but the energy producing material must be of such a kind as to be instantly available. The oxygen supply

is known to be very generous. The fatty acids found in brain tissues are among the most saturated found anywhere, lecithin and related phospholipoids are present in large amounts, hydroxy fatty acids are found in relatively large proportion and compounds of fatty acids with a sugar are found.

Fatty Acid Synthesis.—The naturally occurring fatty acids are all straight-chain compounds of even numbered carbon atoms and containing mainly sixteen or eighteen carbons, facts which point to a two carbon atom origin. Animal fat undoubtedly originates to a considerable extent from carbohydrate and the prevailing hypothesis regarding the transformation is that the carbohydrate is broken down to the two carbon stage, presumably to acetic aldehyde and then by successive aldol condensations is recombined to form the various members of the fatty acid series. Shaffer ⁴⁷ believes that the hypothesis is probably wrong, since at the first step the aldehyde of β oxybutyric acid, a ketogenic substance, is produced—which would make the carbohydrates ketogenic. But of course a ketogenic substance has to be formed at some time if fats are formed. The formation of diacetic acid from acetic acid in glycogen-free livers, noted above, ³⁰⁻³¹ should be considered in this connection.

Abnormalities in Fat Metabolism.—The recognized abnormalities of fat metabolism are few in number and the available information regarding them can be stated in a few words.

Abnormalities in Cholesterol Metabolism.—Cholesterol is practically insoluble in water and its presence in blood, even in tenths of 1 per cent., forms a supersaturated solution which might be expected to deposit cholesterol when conditions were right. Actually deposition does not take place at normal and rarely at higher concentrations. Experimental hypercholesteremia in rabbits produces deposits of cholesterol, mainly in the form of esters, at various points, particularly in the walls of the arteries, and is believed by some to be a factor in the production of arteriosclerosis. The deposition of cholesterol from the concentrated bile in the gall bladder in the form of gall stones is of course well known and some ²² have concluded that there is a causal relation between high blood cholesterol and gall stone formation.

The relation between cholesterol and certain blood diseases, such as the various anemias, has been suggested, but the evidence is not sufficient to warrant definite conclusions.

Obesity.—There is a prevailing popular belief in a certain mystery regarding obesity—that some people become fat on a diet that barely supports others. This belief has led to many experiments to discover if there was any essential metabolic difference between individuals but the results have been mainly negative. The relation between surface area and energy requirement in the resting state holds for all individuals⁴⁸ and each piece of work requires (within narrow limits) a definite expenditure of energy which can be calculated and which applies to practically all individuals in the same way. Laying on fat means in general just one thing—that the energy intake exceeds the energy outgo and can be satisfactorily treated on that basis. There are a few exceptions to this rule, such as adiposity of endocrine origin.

In addition to the disadvantages of carrying around extra weight in the form of fat there are certain dangers, one of these—the tendency of fat people to diabetes—may be either cause or effect, but it is well known that diabetes in a great many cases, perhaps the majority, is preceded by obesity. The laying on of fat in incipient diabetes is perhaps to be explained as follows: there are two paths open to the carbohydrate of the food, combustion and storage, mainly in the form of fat. When the path to combustion is partly blocked through the lack of insulin, the first effort of the organism is to save the carbohydrate by converting it into fat. Later with the increasing lack of insulin the difficulty of burning sugar increases, it accumulates in the blood and then begins to waste in the urine. Finally the wasteage becomes so great that the food cannot supply the energy requirement and the accumulated fat melts away, producing the emaciation characteristic of the advanced diabetic. The tendency of even the severe diabetic to form and store fat is seen by the fact that after the ingestion of levulose the R. Q. often is exceptionally high⁴⁹ and also from the recently reported

work of Richardson and Mason ⁵⁰ that the diabetic tends to burn more protein, more carbohydrate and less fat than he receives.

Acidosis.—The acidosis most frequently present in the animal body is that due to incomplete combustion of the fatty acid chain resulting in the production of the acetone bodies—diacetic and β oxybutyric acids—which are relatively strong acids. As such they must be gotten rid of in the same way as the other strong acids produced in metabolism, *i.e.* by combination with bases and excretion as salts, with the result that in extreme cases the organism is robbed of the fixed bases which are necessary for respiration. There is no doubt that as the supply of available base becomes small the unneutralized acid in the cells may reach a dangerous concentration and the cell be permanently injured, which may explain the failure of alkali therapy in severe acidosis and the general downward progress of the whole metabolism when even slight acidosis is allowed to persist. Whatever may be said regarding the practice of allowing a moderate degree of glycosuria or a moderately high blood sugar with the idea that the greater concentration of sugar in the blood will produce by mass action a greater utilization—a conception which is in agreement with our present knowledge of such balanced reactions—there can be no question that the accumulation of acetone bodies even in small amounts cannot but be harmful even though thereby more is consumed.

Why the oxidation of the long fatty chain should stop at the four carbon stage and that when the initial stage (β oxidation) in the next step of oxidation has already taken place, is an interesting subject for speculation. It is generally believed that the short chain fatty acids are more easily oxidized than the long ones—as witness the belief in the breaking of the long chains. Also diacetic acid is a very reactive substance and so should be more easily disposed of than butyric acid. And yet it is just the stage that cannot be passed without the simultaneous combustion of carbohydrate. A possible reason for the break in the series of oxidations at this point is the increasing acidity of the fatty acids as the chain shortens. The long-chain fatty acids are very weak acids. With the shortening of the chain

the residues become increasingly stronger acids until at the four carbon stage the acid is of significant strength and is still stronger by virtue of the oxygen in the chain. Another factor which may be of importance is the increasing solubility of the residues. The long-chain acids are insoluble in water while butyric acid is relatively soluble and diacetic and β oxybutyric acids still more so. It is possible that the increasing solubility and acidity reach a stage at this point where conjugation with some part of the glucose molecule is necessary to overcome the injurious effect of these properties.

The relation of carbohydrates to the combustion of the fatty acids is an interesting problem and much work has been done on it by Shaffer, Woodyatt, Hubbard and others. It has been, however, the subject of a recent lecture by Shaffer⁴⁷ before this Society and so need not be detailed. The relation appears to be quantitative, one molecule of glucose being effective for the oxidation of two molecules of fatty acid. Under bodily conditions double that amount of glucose is necessary for complete avoidance of acetone body production due, as Shaffer suggests, to difficulties in distribution. His *in vitro* experiments indicate that glucose to be effective must be in process of oxidation. Having in mind the ready reactability of acetoacetic ester or its sodium salt, Shaffer has experimented with various aldehydes in the hope of getting some light on the glucose derivative which combines with diacetic acid to bring about its destruction. His experiments led him to no definite conclusion other than that glucose itself is not the effective substance.

Of interest in this connection is the recent announcement of Intarvin. Following up the early work of Ringer who found that the odd numbered carbon fatty acids did not produce acetone bodies but instead sugar, Kahn and McKee, of New York, have synthesized a fat in which the fatty acids are all long-chain (C 17) odd numbered fatty acids, with the idea that thereby the nutriment of fat could be provided without danger from acidosis. The subject is apparently still in the early experimental stage, for very little information is available concerning it. There seems to be no doubt that the new fats are absorbed and

that no acetone bodies are produced—the acetone body excretion drops at once when feeding with this fat begins and as promptly rises when ordinary fat is substituted—but that all may not be clear sailing is indicated by the fact reported from one laboratory that the organic acidity as determined by Van Slyke's method is scarcely affected by the change and that therefore there may be a replacement of one organic acid for another.

Lipemia.—The blood plasma in the post-absorptive condition is generally clear, or at least nearly free from suspended fat particles. Normally a meal of fat is absorbed into the blood stream and has disappeared again from it in the course of twelve to fourteen hours. A milkiess that persists longer than twenty-four hours after a meal is probably to be regarded as abnormal. Such a milkiess of the plasma may appear in fasting, due to very rapid discharge from the fat stores. It may occur as the result of extensive hemorrhage in some animals as for example the rabbit, where the source is again largely the fat of the stores and probably particularly in this case the bone marrow, although the lipemia is greater when the animal is given fatty food.¹⁴ It is relatively frequent in severe diabetes in which the source is undoubtedly the fat of the food. But no matter what the source, the development of the lipemia and its final form is approximately the same. The lipid constituents increase in the following order, first the fat, then the lecithin and third the cholesterol. As the lipemia persists the cholesterol continues to increase while the lecithin, after reaching a certain value, remains stationary. There is a tendency toward a balance between cholesterol and lecithin, such as exists in fasting plasma but in long-lasting lipemia the greater increase of cholesterol results in a disturbance of the balance.

The occurrence of lipema may, in most cases, be explained as a disturbance in the balance between inflow and outflow of fat. In diabetic lipemia the disturbance appears to be in the outflow, because a high-grade lipemia lasting two or three weeks may sometimes be produced by a single fat feeding,¹³ and in other cases a lipemia of unknown origin may persist for an equally long time on a fat-free or low-fat diet. In fasting and hemor-

raghic lipemia the disturbance is caused by an abnormally large inflow, since the disappearance of the lipemia is rapid. The mechanism of diabetic lipemia has been worked out by Allen⁵¹ in experiments with dogs made experimentally diabetic by removal of a large proportion of the pancreas and then by suitable feeding. The conditions under which lipemia occurred in his animals were two in number, first and apparently most important, was the existence of active, severe symptoms in the form of glycosuria and hyperglycemia and, second, a sufficient supply of fat in the diet. Mild cases even with high glycosuria and severe cases in which the glycosuria has been abolished by diet never exhibit any extreme grade of lipemia, no matter how high the fat intake. The lipemia then represents not a primary disturbance in fat metabolism but is a necessary phenomenon due to the breakdown in carbohydrate metabolism as the result of endocrine disturbance. Wide variations in susceptibility are found in both animals and patients.

Allen's conclusions are compatible with those of Joslin⁵² obtained by a study of blood lipoids in a large number of diabetics. Joslin's findings are that diabetics with lowest metabolic level and most below standard body-weight have the highest blood fat. Apparently any treatment which will improve the metabolic condition of the diabetic will abolish the lipemia. Most remarkable is the fact that feeding of fat to the severe diabetic with lipemia will often cause a disappearance of the lipemia,⁵³ presumably through an improvement in his condition, the fat of the food sparing body protein.⁵⁴

REFERENCES

- ¹ Krogh, A., and Lindhard, J.: *Bioch. J.* 1920, xiv, 290.
- ² Bloor, W. R.: *Proc. Soc. Exp. Biol. & Med.* 1920, xvii, 138.
- ³ Moore, B., and Rockwood, D. P.: *J. Physiol.* 1877, xxi, 58.
- ⁴ Zucker, T. F.: *Proc. Soc. Exp. Biol. & Med.* 1920, xvii, 89.
- ⁵ Joannovics and Pick, E. P.: *Wien. klin. Woch.* 1910, 573.
- ⁶ Lee, F. C.: *Johns Hopkins Hosp. Bull.* 1922, xxxiii, 21.
- ⁷ Bloor, W. R.: *J. Biol. Chem.* 1913-14, xvi, 517.
- ⁸ Terroine, E. F.: *J. de Physiol. & Path. Gen.* 1914, xvi, 386.
- ⁹ Gage, S. H., and Fish, P. A.: *J. Am. Vet. Med. Assoc.* 1921, lviii, 384.
- Neisser and Bräuning, *Zts. Exp. Path. & Ther.* 1907, iv, 747.

- ¹⁰ Meigs, E. B., Blatherwick, N. R., and Carey, C. A.: *J. Biol. Chem.* 1919, xxxvii, 1.
- ¹¹ Bloor, W. R.: *J. Biol. Chem.* 1914, xix, 1.
- ¹² Mayer, A., and Schaeffer, G.: *J. de Physiol. & Path. Gen.* 1912, xv, 534.
- ¹³ Bloor, W. R.: *J. Biol. Chem.* 1921, xlix, 201.
- ¹⁴ Horiuchi, Y.: *J. Biol. Chem.* 1920, xlv, 345.
- ¹⁵ Bloor, W. R.: *J. Biol. Chem.* 1923, lvi, 711.
- ¹⁶ Thiele, F. H.: *Bioch. J.* 1913, vii, 275. Porter, A. E.: *Bioch. J.* 1916, x, 523.
- ¹⁷ (a) Levene, P. A., and Ingvaldsen, T.: *J. Biol. Chem.* 1920, xliii, 359.
 (b) Levene, P. A., and Rolf, I. P.: *J. Biol. Chem.* 1921, xlvi, 193.
 (c) Levene, P. A., and Rolf, I. P.: *J. Biol. Chem.* 1921, xlvi, 353.
 (d) Levene, P. A., and Simms, H. S.: *J. Biol. Chem.* 1921, li, 285.
 (e) Levene, P. A., and Rolf, I. P.: *J. Biol. Chem.* 1922, liv, 91.
 (f) Levene, P. A., and Rolf, I. P.: *J. Biol. Chem.* 1922, liv, 99.
- ¹⁸ MacLean, Hugh: *Lecithin and Allied Substances*, Longmans, Green & Co., 1918.
- ¹⁹ Levene, P. A., and Rolf, I. P.: *J. Biol. Chem.* 1923, lv, 743.
- ²⁰ Wacker, W., and Hueck, L.: *Biochem. Zts.* 1919, c, 84.
- ²¹ MacCallum, W. G.: *Physiol. Reviews.* 1922, ii, 70.
- ²² Henes, E.: *Surg. Gynecol. & Obst.* 1916, xxiii, 91. Rothschild, M. A., and Wilensky, A. D.: *Am. J. Med. Sci.* 1918, clvi, 239, 404, 564.
- ²³ Hürthle, K.: *Zts. f. Physiol. Chem.* 1895-6, xxi, 331.
- ²⁴ Knoop, F.: *Beitr. z. Chem. Physiol. & Path.* 1904, vi, 150.
- ²⁵ Ransom, F.: *Deutsche Med. Woch.* 1901, xxvii, 194.
- ²⁶ Noguchi, H.: *Univ. of Pennsylvania Med. Bull.* 1912.
- ²⁷ Hartley, P.: *J. Physiol.* 1909, xxxviii, 353.
- ²⁸ Lewkowitsch: *Chem. Tech. Oils, Fats & Waxes. I.* Macmillan, 1921, p. 409.
- ²⁹ Schotten, C.: *Zts. Physiol. Chem.* 1882, vii, 375.
- ³⁰ Loeb, A.: *Biochem. Zts.* 1912, xlvii, 118.
- ³¹ Friedman, E.: *Bioch. Zts.* 1913, lv, 436.
- ³² Embden, G., and Loeb, A.: *Zts. Physiol. Chem.* 1913, lxxxviii, 246.
- ³³ (a) Dakin, H. D.: *Oxidations and Reductions in the Animal Body.* Longmans, Green & Co., 1912.
 (b) Dakin, H. D.: *Physiological Oxidations*, *Physiol. Reviews*, 1921, i, 394.
- ³⁴ Dakin, H. D.: *J. Biol. Chem.* 1907, iii, 57.
- ³⁵ Embden, G., Soloman and Schmidt: *Beitr. z. Chem. Physiol. & Path.* 1906, viii, 136.
 Embden, G., and Marx, A.: *Beitr. z. Chem. Physiol. & Path.* 1908, xi, 318.
- ³⁶ Blum, L.: *Münch. med. Woch.* 1910, lvii, 683.

- ³⁷ (a) Ringer, A. I.: J. Biol. Chem. 1912, xii, 511; 1913, xiv, 43.
(b) Ringer, A. I., Frankel, E. M., and Jones, L.: J. Biol. Chem. 1913, xiv, 525, 539.
- ³⁸ Blum, L., and Woringer, P.: Bull. Soc. Biol. Chem. 1920, ii, 88.
- ³⁹ Magnus-Levy, A.: Arch. f. Exp. Pharm. & Path. 1899, xlii, 149.
- ⁴⁰ Mandel, A. R., and Lusk, G.: Am. J. Physiol. 1903, x, 55.
- ⁴¹ Mandel, A. R., and Lusk, G.: D. Arch. f. klin. Med. 1904, lxxxi, 472.
- ⁴² Lusk, G.: Am. J. Physiol. 1908, xxii, 172.
- ⁴³ Geelmuyden, H. C.: Ergebnisse der Physiol. 1923, xxi, 274.
- ⁴⁴ Ringer, A. I., and Lusk, G.: Zts. f. Physiol. Chem. 1910, lxvi, 106.
- ⁴⁵ Dakin, H. D.: J. Biol. Chem. 1913, xiii, 513; 1913, xiv, 321.
- ⁴⁶ Grey, E. C.: Bioch. J. 1913, vii, 148.
- ⁴⁷ Shaffer, P. A.: Harvey Lecture, 1922.
- ⁴⁸ DuBois, E. L.: Arch. Int. Med. 1916, xvii, 887.
- ⁴⁹ Joslin, E. P.: Treatment of Diabetes Mellitus. Lea & Febiger, 1923, p. 296.
- ⁵⁰ Richardson, H. B., and Mason, E. H.: J. Biol. Chem. 1923, lvii, 587.
- ⁵¹ Allen, F. M.: J. Metab. Res. 1922, ii, 219.
- ⁵² Joslin, E. P.: p. 232.
- ⁵³ Marsh, P. L., and Waller, H. I.: Arch. Int. Med. 1923, xxxi, 63. Joslin, E. P.: p. 195.
- ⁵⁴ Woodyatt, R. I.: Arch. Int. Med. 1921, xxviii, 125.

THE CHEMOTHERAPY OF PROTOZOAN AND BACTERIAL INFECTIONS ¹

DR. WALTER A. JACOBS

The Rockefeller Institute for Medical Research

THE term Chemotherapy in its more restricted sense is employed to designate that branch of investigation which deals with the discovery of chemical agents which act specifically on infectious diseases and with the study of their mode of action. The introduction of this term we owe to Ehrlich and the great progress which has been made in this field within the comparatively short space of two decades is due largely to the brilliant achievements and leadership of this worker. The subject is now so vast that in the short time which is allotted to us we cannot do more than discuss certain phases of what has been learned. Our treatment of the subject must naturally fall into two divisions, that dealing with the chemotherapy of bacterial infections and finally, that in which animal parasites have been made the objective. We shall deal with the former first, since efforts to influence experimental bacterial infections with chemical compounds antedate similar studies with animal parasites.

Not long after it became known that there are substances which would kill micro-organisms in the test tube, attempts were made to apply such germicides to disinfection within the organism. All of these experiments postulated a direct action of the chemical upon the micro-organism within the host such as occurs in disinfection outside of the body. Among the very first investigations in this direction were those of Koch ¹ who attempted to sterilize animals which had been infected with anthrax bacillus by the use of bichloride of mercury.

Although he was able to inject this substance into the blood in such an amount as to achieve a concentration many times that which was necessary to kill the micro-organisms *in vitro*, all

¹ Delivered December 8, 1923.

the attempts proved fruitless. Koch attributed the discrepancy between the *in vitro* and *in vivo* results to the fact that mercuric chloride forms a chemical union with the serum proteins, as evidenced by the formation of a precipitate and was therefore rendered ineffective.

In later work, particularly that of von Behring, we see numerous other attempts to achieve internal disinfection. Occasionally indications were obtained with the use of certain inorganic substances but such results proved of no practical value because of their great uncertainty and irregularity and the great toxicity for the animal shown by the amounts of chemical required. Although occasional efforts in this direction continued, this type of investigation came to be regarded as futile and almost impossible of achievement. We can see in the words of von Behring an expression of this gloomy outlook. After ten years of persistent attempts he finally concludes: ²

“It can be regarded almost as a law that the tissue cells of man and animal are many times more susceptible to the poisonous effects of disinfectants than any bacteria known at present. Therefore, before the antiseptic has a chance either to kill or to inhibit the growth of the bacteria in the blood or in the organs of the body, the infected animal itself will be killed. The pessimism of him who declared that disinfection in the living body is for all time impossible appears to be only too justified.”

But with the more recent highly successful application of synthetic drugs to the treatment of protozoan diseases and because of the failure in so many instances of the more rational immunological methods, interest again centered in the possibility of the therapeutic application of bactericidal agents. The problem has proven extremely difficult to approach by any other means. It has been particularly difficult because of the lack of an empirical background such as had placed the qualities of arsenic, quinine and mercury at the disposal of the worker with protozoan infections. It has seemed to some that the only method short of the mere empirical testing of all sorts of substances in the experimental animal was to employ the

bactericidal test at least as a means of initial orientation, even though it appear the crudest makeshift.

The first extensive experiments in this direction were made by Bechhold³ and Ehrlich, who found in the group of halogenated phenols a number of substances which exhibited *in vitro* a high order of inhibiting action on the growth, especially of the diphtheria bacillus. At the same time it was possible to follow certain influences which chemical constitution seemed to exert on antiseptic action, such as the exalting effect, up to certain limits, of the multiplication of halogen groups or the addition of methyl groups to the molecule. But what is more important was the observation that none of these substances would sterilize laboratory animals infected with the diphtheria bacillus, and this, in spite of the fact that the toxicity of some of the substances was low enough to permit the achievement of a concentration in the blood 100 times that required to kill this bacillus in the test tube. A possible explanation for this failure was again found in the greater affinity of these disinfectants for the blood proteins than for the bacteria. Although no visible precipitate was caused on mixing their solutions with serum, as in the case of mercuric chloride, their bactericidal action *in vitro* was greatly reduced by the process. In this work we find for the first time the expression "partial specificity" to designate a type of selective antiseptic action which a chemical may display towards a particular micro-organism.

Compatibility with serum and partial specificity are factors upon which the subsequent workers with antiseptics have come to lay particular emphasis and their observations have furnished us with not a few examples of the latter. In the tendency toward specificity was found a grain of hope, since it seemed a step in the right direction—in the direction of the specificity displayed by immunity principles. Many instances of partial specificity have been observed especially among the organic dyes. A number of these observations have been used as the basis of methods for the differentiation of bacteria in culture, such as that which employs gentian violet in media to restrain the growth of Gram-positive strains, or the use of brilliant green

to separate the typhoid from the colon bacillus. A study of the bactericidal properties of the quaternary salts of hexamethylenetetramine⁴ has brought out instances of substances which displayed a decided preference for the streptococcus. In not a few cases, substances were found which killed the streptococcus in dilutions which were 20-30 times greater than those required to sterilize suspensions of meningococcus and gonococcus. A most unusual example of specificity has come to light in the case of the toxicity of the alkaloid optochin for the pneumococcus, but to this we shall return later. Quite recently Browning, Cohen and Gulbransen,⁵ have uncovered perhaps a still more remarkable instance in the case of the cyanine dye, sensitol red. The lethal effect which it displays against staphylococcus is 2000 times that which it exhibits against the colon bacillus in peptone water. I am, however, not aware of any experiments which have been performed with this substance in infected animals.

In the quality of serum compatibility we have presented to us in its simplest form the idea that a substance must display a greater affinity for the micro-organism than for the tissues of the infected animal in order to be germicidally efficacious. But few substances have been found which retain their full activity in the presence of serum. A few favorable instances have been observed among the quaternary salts of hexamethylenetetramine which were prepared from certain chloracetyl-amino compounds.⁴ A number of these exhibit a bactericidal effect in serum and protein solution which is very little less than that found in salt solutions, but their application to internal disinfection was without result. Optochin maintains toward the pneumococcus a high degree of bactericidal efficiency in serum. The low concentration of 1:20,000 in serum and even that of 1:8000 in oxalated blood have proved sufficient to kill this micro-organism in 2 hours and in much higher dilutions it exhibits a definite growth restraining influence.⁶ However, this is considerably less than what is observed in ordinary salt solution, since in the latter medium a dilution of 1 to 500,000 or more is all that is required. Much more favorable relationships have been observed in the case of acridine compounds. Browning

and his co-workers⁷ have found that diaminoacridine and its methochlorides, or proflavine and flavine respectively, actually exert a more potent action on the staphylococcus and colon bacillus in serum than in ordinary saline. This very unique quality and the unusually potent bactericidal properties of these substances led to their application in the local treatment of wound infections.

Still more difficult than to find substances which will resist the diverting influence of serum constituents is to find antiseptics which will maintain a high bactericidal effect in blood. In experiments in our laboratories, Doctor Felton⁸ made a number of observations in regard to this point, and was able to find but few instances of substances which retain a high order of antiseptic potency in this medium. Mention may be made of malachite green and a few of its derivatives and especially the flavines and certain dyes of the rosinduline group. The observed values were, however, lower than those shown in ordinary saline.

There are still other properties which may be considered among the desiderata for the ideal internal disinfectant. One of these is speed of bactericidal action. It has been very difficult indeed to find substances which act rapidly under the conditions which obtain in the circulation. In order to kill the pneumococcus in 15 minutes, optochin must be used in a concentration of 1:1000 in whole blood. Even flavine in the same concentration fails to sterilize in one hour, although in two hours it may act in a dilution of 1 to 32,000. Taken alone, speed of action may not be essential, especially if a substance can be maintained sufficiently long in the circulation to develop slowly its action or to exercise merely a growth restraining or bacteriostatic effect. But here again we must postulate other conditions in order to obtain such a result. Repeated observations with optochin in animals and in man have shown that it is possible to render the blood antiseptic for pneumococcus but the observations of Moore and Chesney⁹ have shown that this may be complicated by the development of fastness. Browning and Gulbransen¹⁰ have demonstrated that the blood of rabbits may

display a degree of inhibiting action for staphylococcus and the colon bacillus for several hours after the administration of diaminoacridine, but this alone was apparently inadequate.

In the control of certain infectious processes the difficult requirements of proper distribution and tissue penetrability of the antiseptic must be taken into consideration. In a former lecture before this Society, Lewis, in describing his experimental studies on the chemotherapy of infections produced by the tubercle bacillus, has stressed the importance of combining in a substance a degree of germicidal action with specific tissue penetrating power.

Finally, there is another group of requirements which must be discussed in connection with the *in vivo* operation of bactericidal substances. Not only must a substance be of sufficiently low general toxicity, but it must prove comparatively harmless to the general powers of resistance and to the normal protective mechanism of the infected animal. In this connection we may have to deal with quite an extensive and perhaps less tangible group of factors which can be incorporated into systematic study only with the greatest difficulty. It may be just at this point that the real difficulty lies and we shall have occasion to refer to this later.

In this connection, observations have been made to determine the effect on phagocytosis which might be exhibited by bactericidal substances. The experiments of Felton⁸ have recently shown that not a few highly bactericidal dyes and cinchona derivatives markedly reduce the phagocytic index in dilutions in which they might exhibit a bactericidal effect in blood. Exceptions in this regard may perhaps be the flavines, which, according to Browning, are not appreciably injurious in dilutions above 1:500.

But if we consider in retrospect all of the conditions which may separate the observed *in vitro* properties of an antiseptic from its successful operation in an infected animal, it is apparent that we have presented a very difficult case for the idea that the control of bacterial infections may be approached from the standpoint of internal disinfection. This has certainly been

very generally borne out in attempts to apply such substances in actual infections. We shall later see that even in the more successful application of compounds to the control of protozoan infections, there have appeared repeated discrepancies between the action of substances on the parasite in the test tube and in the animal. It would seem, therefore, that for the time at least, we are thrown back on the empirical testing of substances in the infected animal as the only safe procedure.

The first striking instance of the successful application of a chemical to the treatment of a bacterial infection in the laboratory animal we owe to the observations of Morgenroth and his co-workers. Previous studies of Neufeld¹¹ and others had shown a certain biological similarity between trypanosomes, spirilla and the pneumococcus in the susceptibility of their envelopes to the dissolving action of bile salts. While engaged in a study of the effect of quinine derivatives in experimental trypanosome infections in small animals, Morgenroth and Halberstadter¹² were able to demonstrate a certain curative effect of these substances. Then partly because of the possible biological similarity between the pneumococcus and the trypanosome and because of certain clinical observations on the applicability of quinine in the treatment of pneumonia, he was induced to make similar studies in experimental infections with the pneumococcus. Morgenroth and Levy¹³ found that a pneumococcus septicemia induced in mice was but little affected by injections of quinine. On passing to hydroquinine, which differs from it by but two hydrogen atoms, a more definite influence was to be seen in delaying the death of the animals. An optimal effect, however, greatly exceeding that of the former was furnished by ethylhydrocupreine, or optochin, in which the methoxyl group of hydroquinine has been changed to ethoxyl. It was found possible to save a good percentage of the infected animals by repeated injections particularly of a solution of the drug in oil. Unfortunately the clinical application of the drug has been limited by its untoward toxic properties, especially the tendency to produce amblyopia. Moore and Chesney,⁹ from extensive observations on patients suffering from acute lobar pneumonia, have

concluded that the use of optochin in the treatment of this condition cannot be recommended, since it is impossible to administer a sufficient amount to produce an effective concentration in the blood stream without exposing the patient to the danger of toxic effects. During the use of the necessarily subeffective doses the pneumococci were observed even to become fast to the drug.

It would appear that Morgenroth in his first experiments was unaware of the potent germicidal action of this substance on the pneumococcus, so that a knowledge of this property played no part in its initial chemotherapeutic application. We owe to Wright¹⁴ the recognition of this quality as well as that of the fact that optochin kills the pneumococcus in high dilution in the presence of serum. The confirmation of these observations led Morgenroth to attribute the curative action of optochin in the animal to its direct bactericidal effect and in his later studies he used the test tube as a guide. This later work¹⁵ developed the interesting influence which the chemical structure of the cinchona alkaloids exhibited on their specific anti-septic action.

On passing from quinine, through hydroquinine to optochin, a sudden jump in the action upon the pneumococcus occurs to fall again on passing higher up the series of alkyl compounds. However, with the stage of the isoamyl ether a bactericidal effectiveness towards streptococcus and staphylococcus becomes evident which is augmented on passing to the heptyl and isooctyl compounds and then drops again. These observations while interesting in themselves had to do rather with external disinfection and proved inapplicable to the treatment of systemic infections.

It would seem that the powerful bactericidal properties of optochin lend support to the belief that its curative effect in mice is a result of its direct bactericidal action. But certain observations of Neufeld and Engwer,¹⁶ and later by Moore,¹⁷ have suggested the interposition of the host in the process. Moore found that a single small dose of optochin in oil which in itself was quite ineffective in the treatment of an experimental pneumococcus septicemia in the mouse, when combined in the

treatment with an amount of homologous anti-pneumococcus serum which was also far under the effective dose was capable of increasing the threshold value of this serum at least fifty times. This result indicates that the combined action of the two therapeutic agents was many times that of a simple summation of their protective effects. Whether a similar synergism or rather fortification occurs between the drug and certain undetermined protective factors when the drug is used alone in a so-called curative dose has not been definitely ascertained, but Moore's observations would seem to weaken somewhat the view that the bactericidal effect is the only factor.

Taking all that we have considered with reference to optochin and its near relatives, as well as the ancient reputation which quinine has always had, we cannot escape the enticing suggestion that perhaps there is something inherent in the molecule of these alkaloids which, like the elements arsenic and mercury, has conferred upon them certain potentialities as specific etiological remedies and that this property might be turned to wider usefulness if we could only find the way.

A number of years ago we turned our attention to this problem in the laboratories of the Rockefeller Institute at the suggestion of Doctor Flexner and also of Doctor Cole who was particularly interested in the possibility of finding new therapeutic agents for the treatment of infections produced by the pneumococcus. In this work Doctor Heidelberger and I were extremely fortunate in the enjoyment of the skilful biological coöperation of Doctor Wollstein and later of Doctor Felton.

The first problem was purely chemical. We have already seen what a pronounced change in biological properties has followed but slight chemical alterations in the quinine molecule. Would it be possible to find other means of altering the cinchona molecule in a systematic manner, but in a way to maintain its general integrity, and perhaps permit us to follow the influence of chemical modification on biological action? The naturally occurring members of the group, as we have seen, had been thoroughly studied and also their hydro-derivatives and the homologous ethers of hydroquinine as well. A successful solu-

tion of the chemical problem was found by making use of the fact that these alkaloids as tertiary bases react with alkylhalides to form quaternary salts.¹⁸ In one direction in particular—by the use of chloroacetyl amino compounds as alkylhalides it was possible to develop if necessary a practically unlimited number of new derivatives, and, in a way, to leave the original alkaloid molecule intact. Many substances were prepared into which a thorough selection of the usual organic groups had been introduced. Then again a similar opportunity was offered in the preparation of a series of hydroxyazo dyes from hydrocupreine,¹⁹ the phenolic compound obtained by demethylating hydroquinine. By coupling hydrocupreine with the diazonium salt of any aromatic amino compound it was possible to make a logical series of substances in which any chemical change could be featured at will. Still other series,²⁰ of a similar character were prepared, so that in a way it might be said that the opportunity of testing the influence of chemical changes on the action of these alkaloids had been fairly completely presented. While realizing from all that was said before that the measure of bactericidal power was of doubtful importance, nevertheless, such studies were made because of their possible statistical value and since no other criterion short of the animal test was available. Within the groups of quaternary salts and azo dyes numerous instances of marked bactericidal power for the pneumococcus were noted and in several cases were found to equal even that of optochin. However, in the majority of instances, this activity was found to be greatly diminished in the presence of serum. I shall not burden you with an extensive account of these observations, but shall rather summarize the results obtained in the use of these substances in the experimental pneumococcus infection. Such tests made on substances, irrespective of bactericidal potency, showed a few which seemed to exhibit a small measure of therapeutic effect. The quaternary salts of hydroquinine with chloroacetyl-p-aminophenol and chloroacetyl-p-anisidine, in particular, if given not too long after inoculation of the mice or if not in too large amounts, often, though irregularly, cured the animals. An interesting and not infrequent observation which

was made with these and similar substances was the effect of doses above a certain threshold, even though much under the lethal dose. In such cases, instead of the apparent cure observed with smaller doses, an actual acceleration of the disease was noted. An explanation of this curious paradox is perhaps not difficult to find, and may lie in the possibility that amounts of drug which are not frankly toxic may be sufficient to injure the animal's natural powers of resistance in such a way as to make it more susceptible to the infection.

In this connection, we have made also a survey of the various groups of organic dyes in regard to their curative effect on pneumococcus infections in laboratory animals, but with similar unpromising results. Even flavine with its high bactericidal power in blood and serum for the pneumococcus gave but the slightest indication of influencing the infection. Observations made with quite a variety of other classes of substances were of a similar negative character.

To sum up—while the experience of workers has brought to light not a few groups of bactericidal compounds, very few individual substances have displayed even a trace of specific therapeutic activity. These few substances have proved to be unique within the group to which they belonged, since closely related compounds, or those differing from the active substances by relatively insignificant chemical modifications have proved to be quite useless. I shall not keep you longer with this portion of the story except a word perhaps as to the general outlook for the chemotherapy of bacterial infections. At present the data available seem quite barren of suggestion. But I do not wish to imitate here quite the gloomy view which was quoted at the beginning of this paper. The mere instance of optochin and of the therapeutic value of chaulmoogra oil and the more recently used esters of chaulmoogric and hydnocarpic acids obtained from this oil in the treatment of leprosy are indications that the control of bacterial infections by drugs is by no means impossible of achievement. The real difficulty lies in the necessarily opportunistic experimental method and the lack of a rational scientific means of approach. The problem is essentially a search for a

substance of unknown nature but which must yet perform a service by a mechanism of which we are equally uncertain. It may be possible to inquire successfully into what biological properties such a substance must possess, or under what biological conditions it will have to operate; but such information will not point to the substance itself. In the case of bacterial infections, experience has presented us with too few points of contact between our knowledge of chemical structure and whatever biological properties may be required in the exhibition of a therapeutic effect to present a rational basis for the search for curative substances. Perhaps the real solution of the problem may be ultimately found by gaining an insight into the chemical nature and mode of operation of the substances which are normally elaborated by the organism in its fight against infection.

If we turn now to the application of chemical agents to the treatment of protozoan diseases, we are confronted by a far more encouraging state of affairs. We have already alluded to the traditional virtue of quinine and mercury and that these were purely empirical acquisitions. However, with the recognition of the etiological factors of the diseases which these substances influenced, the role which they occupied as real curative agents was likewise recognized. During the past twenty years considerably more has been added to our chemical equipment in the treatment of this group of diseases as a result of the application of laboratory methods of both chemistry and biology. In the present paper I can, however, touch upon only certain phases of this development. The appearance in 1902 of the classical experiments of Laveran and Mesnil,²¹ was the beginning. By the use of sodium arsenite these workers succeeded in temporarily clearing the blood of mice infected with the parasite responsible for the disease of cattle called "Nagana." Then there followed within a few years a succession of observations in which substances belonging to a number of different groups were found to possess a similar efficacy in experimental infections produced by the same or similar animal parasites. These substances fall naturally into several groups which it will serve our purpose best to treat separately. Soon after the observations

of Laveran and Mesnil, Ehrlich and Shiga,²² demonstrated that mice infected with *trypanosoma equinum* could be permanently cured by a single injection of trypan red, a tetrazo dye prepared by coupling tetrazotized benzidinesulfonic acid with a naphthyl-aminedisulfonic acid. This is noteworthy as the first instance of the cure of an experimental trypanosome infection with a single dose of a synthetic chemical. However, the value of the substance itself seems to have been limited, since it failed to exert a similar therapeutic effect in other animals or with other types of trypanosomes.

We meet here for the first time a peculiar paradox which was destined to appear repeatedly in later work. Although trypan red proved to be active *in vivo*, it was inactive in the test tube. At the time, these workers noted the staining of the tissues of the animal by the dye after injection. They considered that its action was possibly due to the persistent effect of small amounts of the drug or of some active alteration product of it upon the parasites. The production of an active immunity in the animal by the rapid destruction of the parasites by the drug was considered also as a contributing factor. Observations of a similar character with other dyes became the basis for much subsequent discussion and inquiry as to the mechanism involved in the successful removal of trypanosome infections by such substances. In these discussions, which I cannot present at length, attempts were made to explain this paradoxical action also by the assumption of a direct injury produced by the dye on the trypanosome of a character which did not affect their motility but prevented their multiplication. The suggestion that a chemical alteration of these substances in the body to a more active form, while not so obviously applicable to this class of substances, has been more successfully applied, as we shall see, in explaining the action of arsenic compounds. Subsequently, Nicolle and Mesnil,²³ found, from the study of a large number of similar substances, a benzidine dye trypan blue which proved still more efficacious in that it cured mice infected with the more resistant trypanosomes of Nagana and Surra. Dyes of all kinds were then investigated and, in succession, members

of the triphenylmethane group and a number of orthoquinoid dyes such as the pyronines, oxazines, thiazines and acridines were successfully brought into the field of investigation. But the practical application of the dyes, it would seem, has been on the whole rather restricted. The discovery of the therapeutic properties of the individual members of the different groups appears to have been for the most part the outcome of a rather opportunistic selection of substances. There have been attempts to correlate the therapeutic properties of some of them to their chemical constitution and to their selective staining property for the parasite. But, on the surface at least, it would seem that but few facts have emerged which could be applied to a systematic attempt at their further chemical development, or in explanation of whatever degree of therapeutic value they may have shown.

An exception to this, perhaps, is the rather striking property which was found by Ehrlich and his associates to accompany the presence of the orthoquinoid structure of certain dyes. These dyes quickly produced tolerant or fast strains of trypanosomes which were also quite fast to arsenic. Paraquinoid dyes did not show this effect. Again Nicolle and Mesnil,²³ in a thorough survey of the tetrazobenzidine dye group concluded that in order to develop therapeutic qualities the coupler used must be a naphthalene derivative containing at least one amino group and two sulfonic acid groups. You will see from the chart that this condition is satisfied by both trypan red and trypan blue. And it is possible that this may have been made the starting point for the preparation of a substance known as Bayer-205, which has recently come to offer considerable promise. The exact nature of this substance has been carefully withheld but there is a suspicion that it belongs to a group of compounds which the Bayer Company patented some time ago.²⁴ I have on the chart formulæ which suggest the possible relationship of this substance to trypan red and trypan blue.

Preliminary observations of Händel and Joetten²⁵ and Mayer and Zeiss,²⁶ have shown that this substance possesses remarkable curative properties in experimental trypanosomiasis. In small

doses which were often far below the toxic dose for the particular animal, the drug was found to cure the ordinary laboratory animals which had been infected with different strains of trypanosomes. Here again we find the curious circumstances that the substance itself displayed no marked action on the trypanosomes *in vitro* and microscopic studies seemed to suggest that the drug may act by preventing multiplication of the parasites. Later, therapeutic experiments in dourine in horses pointed to its usefulness as a prophylactic and curative agent but more important was its application to human trypanosomiasis. We shall return to a discussion of this in another connection. Although we can only infer the probable nature of this remedy, it would seem that an advance has been made by applying chemical knowledge to the problem of varying a group of substances which had already given indications of therapeutic values. Thus, the dyes from which this substance has emerged have come to share again, for the time at least, a prominence which was taken from them very early in chemotherapeutic studies by the advance in the application of arsenic compounds.

If we turn now to the arsenic group we find that the organic arsenicals have proved a most profitable direction for the experimental studies which led to the finding of synthetic etiological remedies. This is attributable in part to the fact that we are dealing with substances in which the therapeutic quality is apparently inherent in the arsenic itself irrespective of the actual form or by what mechanism its action may come into play. This has made chemical reasoning simpler in attempting to refer biological action to chemical constitution, for it is far more difficult to interpret what should be the point of reference when a large organic molecule is considered with regard to any inherent biological activity.

The impetus to investigation in this direction came with the report of Thomas,²⁷ that a proprietary arsenic preparation called atoxyl displayed a definite therapeutic effect in animals infected with different strains of trypanosomes. The rapid confirmation of this observation soon led to its employment in African sleep-

ing sickness, and it has proved helpful in the treatment of the early stages of this disease.

But a step of the most far-reaching importance was Ehrlich's brilliant recognition of the true nature of atoxyl as the sodium salt of p-aminophenylarsonic acid or arsanilic acid.²⁸ In analogy with sulfanilic acid, it is formed by the fusion of aniline with arsenic acid. In its essentials, the chemistry of organic arsenic compounds had been pretty thoroughly studied by Michaelis and his co-workers, but the methods at the disposal of these chemists were, in a sense, limited. The discovery that arsenic could be introduced into the molecule by direct arsenation opened to Ehrlich and his associates a means for a most thorough development of the chemistry of this group of substances. The immediate suggestion which the structure of p-aminophenylarsonic acid presented was detoxification by acetylation of the amino group. But the resulting product arsacetin, though possessed of a degree of therapeutic power, was soon discarded because in its clinical application, like atoxyl, it not infrequently produced blindness and other toxic manifestations. Here again the peculiar paradox appears that the pentavalent compounds are without appreciable action on the trypanosomes *in vitro*. But Ehrlich's²⁹ fertile imagination soon found a possible solution for this discrepancy. The fact was noted that individual animals treated with certain pentavalent compounds would show a varying tolerance for the drug and those which exhibited the lower tolerance could be cured by smaller doses. This observation was coupled with the well known ability of the tissues to exhibit a reducing action and with the fact that certain trivalent forms of arsenic are far more toxic than the pentavalent forms. Ehrlich concluded that the pentavalent arsenicals are reduced by the tissues and unfold their activity in the trivalent form. This view was supported by the direct comparison of the relative toxicities of the three stages of oxidation of arsanilic acid, p-aminophenylarsin oxide is 75 times more toxic for the mouse than arsanilic acid, while the arseno compound is 30 times more toxic. Similar toxicity relationships have been repeatedly observed.

But more important still is the contrast in the trypanocidal

effect of the oxide and the arsonic acid *in vitro*. In a dilution of 1:100,000 the former kills the microorganisms immediately, whereas atoxyl fails to act even in a 5 per cent. solution. In some cases the arseno-compounds have shown a similar potent action in the test tube but this has not always appeared. Mention may be made of observations of the failure of salvarsan to act directly upon the relapsing fever organism³⁰ or on the treponema pallidum in the test tube although the organisms thus treated were observed to lose appreciably in virulence as shown by subsequent injection into animals. Ehrlich and Roehl³¹ placed particular emphasis on the oxide stage which may be formed either by reduction of the pentavalent compound or by oxidation of the arseno-derivative as the form in which the biological manifestations eventually appear. It is perhaps important to mention this point, since recent statements have appeared which seem to overlook this fact.

The conception of the biological potency of trivalent arsenic was at once applied to further synthetic attempts to find new curative agents in the arsenic group. The great toxicity of the arsenoxides, however, and the possibilities which the arseno compounds came to afford was the reason for the main interest which seemed to attach to the latter group. This work, as you all know, culminated in the brilliant discovery of the value of salvarsan and its derivatives in the treatment of certain spirochæte diseases.

In his theoretical considerations to explain the action of chemotherapeutic agents, Ehrlich assumed a direct action of the substance or its alteration product on the parasites. He conceived this action as being brought about by the avidity of certain groups or chemoceptors in the cell of the parasite for certain groups contained in the chemical. By assuming these discrete affinities within the cell the attempt was made to give more visible expression to certain phenomena which were observed in the reaction of microorganisms to chemicals containing certain groups in the molecule. But, on the whole, workers no longer agree that such precise definition can be given to the relationship between chemical constitution and therapeutic action.

The efficacy of salvarsan was attributed in part to its ortho-aminophenol group which was supposed to direct the drug at the parasite in a way to permit the lethal action of the arsenic to develop. The analogy was drawn with trypan blue which likewise possesses an amino and hydroxyl group in positions ortho to one another. But there are many cases where such analogies do not hold. It is said, for instance, that the isomers of salvarsan containing this configuration are all much less active than salvarsan itself. Again particular stress has been laid on the dystherapeutic effect of the methyl group but numerous instances have arisen in which substances containing the methyl group have actually exceeded in effectiveness the unmethylated compound. In this respect, tryptaflavine is more efficacious as a trypanocide than the unmethylated proflavine. Quinine, which is methyloxycinchonidine is more efficacious in malaria than cinchonidine or the phenol, cupreine. The sulfuric group is still another well known example. In many combinations it may destroy therapeutic efficiency. Morphine or quinine when converted into the sulfuric esters, although again easily recoverable by saponification, are physiologically practically inert. On the other hand, several sulfuric acid radicals are contained in the molecule of trypan red, trypan blue and probably Bayer-205. The conclusion is forced, therefore, that we may attribute only the bare possibility of certain effects to certain groups in the molecule and in any event, the molecule as a whole certainly must be considered.

But the striking changes in biological properties that often follow very minute changes in structure and the occasional appearance of regularity of biological response to such changes have always been a fascination and a source of temptation to the worker to try to utilize such general tendencies in the synthesis of drugs. Several years ago, a group of us at the Rockefeller Institute, Doctors Brown and Pearce and Heidelberger and myself were tempted to take up a phase of this question. In this work the attempt was made to obtain some information from a systematic biological study of the toxic and therapeutic properties of certain types of arsenic compounds which might

be applied to the finding of new remedies. This work which at first embraced a study of the treatment of experimental trypanosomiasis was later extended to include that of the experimental infections produced by the relapsing fever organism and experimental syphilis in the rabbit.

We have already described the emphasis which has been placed on trivalent arsenicals. Since the biological activity was an exhibition of a property of some form of trivalent arsenic, it was undoubtedly a logical step, if considered alone, to use this group of compounds directly. But we may regard this question from another angle. There seems fair evidence at hand that the greater tolerance for pentavalent arsenicals as a group is not alone a function of their greater biological inertness as compared with the trivalent-compounds but also to the speed with which the greater portion is excreted before the reducing action of the tissues can manifest itself. This is but a manifestation of the fact that the arsonic acids which form neutral salts possess a relatively greater portability in the blood stream and body fluids than the trivalent forms as well as a greater penetrability and may readily and quickly diffuse through the tissues. The arsenoxides possess this to a much less degree and because of their degree of unsaturation are chemically very reactive and while perhaps initially portable, readily become bound by and denaturize such functional or tissue elements as may be determined by the general character of their molecule before the same degree of distribution can be achieved. This rapid fixation finds expression in the rapid exhibition of potent toxic manifestations. The arseno compounds as a group possess double the molecular weight and, although perhaps also quite reactive, are the least readily transported and the least diffusible of all. Where there is no special acid salt forming group, substances of this class may be held in solution for a short time only, aided perhaps by the protective serum colloids, but may be readily deposited as such along the route before complete distribution is possible and then more slowly unfold their toxic effects through oxidation to the oxides. If we were dealing with an infection which is confined to the blood stream alone it would seem, therefore,

admissible to place reliance upon the direct quick action of the trivalent compounds and the simple formula of the ratio of parasitotropic to organotropic properties of a substance. But when the more usual tissue infection is to be influenced the question of distribution and diffusibility becomes a very important factor to consider. It is quite conceivable that certain advantages in this regard may result by the use of the pentavalent form which may compensate for what it may seem to lose in immediate direct parasitocidal properties. By more complete distribution and tissue penetration it might be possible to reach localities which may harbor such residues of an infection as may again become generally active. In such localities the reducing action of the tissues may supply a small but sufficient amount of therapeutically active material.

In spite of Ehrlich's somewhat orthodox adherence to the trivalent idea we can see evidence of his occasional return to the pentavalent compounds. In his *Schlussbetrachtungen*,³⁰ the possibility is pointed out of the applicability of hydroxyaminophenylarsonic acid, the pentavalent stage of salvarsan, for local therapy where the arseno compound circulating in the blood cannot reach the affected tissues in sufficient amount, as, for instance, in local eye involvement. Hata's experiments showed several pentavalent compounds to exhibit a good ratio of curative to toxic action but we obtain the impression that these workers were perhaps prejudiced against the group because of a neurotropic tendency which a number of these substances displayed. This exhibited itself in the development of incoördination in the so-called "dancing-mice."

Considerations of a practical character have arisen in connection with the arseno compounds caused by certain inherent difficulties which are associated with their chemical and physical properties. They are unsaturated, highly reactive substances. Slight modifications in the method or conditions of preparation may have a great influence on their general toxic and also therapeutic behavior. It is not possible to obviate this readily by the subsequent use of the ordinary methods of purification such as recrystallization but very careful control of preparative con-

ditions is required which can be only empirically ascertained. It has been necessary, therefore, to maintain a constant biological control of those substances of the group which are clinically employed. Further, the development of this class of substances is in a sense limited because the arseno compounds depend for solubility on the presence of salt-forming groups. The pentavalent compounds possess much more favorable chemical and physical properties. The arsonic acids as a rule are perfectly stable, crystalline compounds which can be readily purified. They form stable salts and therefore do not require the presence of other salt-forming groups for solubility.

It appeared to us, therefore, that the development of pentavalent compounds was certainly worthy of further trial. In the selection of the chemical material for our studies such groups of substances were chosen which would combine a sufficient degree of accessibility with the opportunity for ample and logical chemical development in such direction as the biological result might indicate. From this material it was hoped to accumulate statistical data regarding the influence of chemical constitution on biological action which could be utilized in further work. An idea of the chemistry of these groups of substances is best obtained by reference to the chart.³² The studies were not alone confined to the pentavalent group but in certain instances where the presence of a solubilizing group would permit, reduction to the trivalent stage was tried to test the value of such a chemical transformation.

Over 230 substances were prepared. Their action as regards toxicity and therapeutic properties in one or more laboratory animals infected with the different animal parasites was studied. The observations were too extensive to attempt to do more here than very briefly and superficially summarize the results. Substances were observed which varied all the way from those possessing a very high toxicity to such which were easily tolerated and at the same time exhibited varying degrees of therapeutic power. But the observations with these substances brought out the fact that in the biological measure of each compound so many different factors had to be considered that it became ex-

tremely difficult to find any sharp regularities with regard to the influence of structure on action. But in some cases, it was possible to note a few very general tendencies which were utilized to advantage in the work.

The most important group and that which was most fully studied was that of the amide and substituted amides of phenylglycine-p-arsonic acid. This type of compound was chosen partly for the reason that the glycine amide NHCH_2CONH group not only offered an excellent chance to add practically any chemical group to the molecule which was desired, but because the glycine amide group itself is a normal constituent of proteins and should be inherently compatible. These substances were in general prepared by the interaction of the amino group in arsanilic acid or its analogues with chloroacetyl derivatives of amines in which members of both the aliphatic and aromatic series were given ample representation. Changes in both therapeutic and toxic effects were observed in response to chemical alterations in the molecule often of a decided character which depended not only upon the animal used but also the type of infecting organism. Only in certain instances was it possible to follow any general tendencies. To begin with, the parent substance, phenylglycine-p-arsonic acid, we have met a near relative before in the form of arsenophenylglycine, which is obtained from the arsonic acid by reduction. Contrary, however, to the potent therapeutic properties exhibited by arsenophenylglycine in experimental trypanosomiasis, the pentavalent form, while it can be tolerated by laboratory animals in large amounts, is practically devoid of therapeutic effect. In this series, as far as it was carried, the carboxyl group, while acting fairly constantly as a detoxifying influence, was found to possess a dystherapeutic effect. This was at variance with instances noted in other groups of compounds and like all such irregularities, which have made prediction impossible, there is no explanation at hand. In general, it has become evident that the tendencies which are observed in the apparent influence of constitution on biological action are confined pretty narrowly to substances which are built on the same general plan.

On passing to the amide or ureides of the compounds containing these carboxyl groups, no matter where they were situated in the molecule, there was in general an appearance of therapeutic effect in spite of a tendency to increased toxicity. This therapeutic effect of course varied greatly with the particular compound, the animal and the infection used. A number of substances of the series from the biological standpoint proved to be of outstanding interest. In an extensive series of biological tests, Doctors Brown and Pearce³³ definitely established that one of these in particular possesses unusually favorable biological properties. This substance, the sodium salt of phenylglycinamide-p-arsonic acid, or tryparsamide, as it has since been named, has now come to be of definite, practical, therapeutic importance.

Tryparsamide is a white, crystalline, stable substance which is extremely soluble in water with the formation of a practically neutral solution and is very easily prepared by the interaction of sodium arsanilate and chloroacetamide. As regards the biological properties of this substance, I cannot do better than quote from Doctor Pearce a sort of summary:³⁴

“Toxicological experiments demonstrated that the reaction of different species of laboratory animals to the drug was of a favorable character. The substance lends itself well to almost any method of administration and can be given to animals in large doses and the toxic effects were confined to doses relatively close to the minimum lethal dose. The recovery of animals from sublethal intoxication was remarkably rapid and complete, thus making possible the repetition of large doses at comparatively short intervals of time. The therapeutic activity of the drug in experimental trypanosomiasis was particularly evidenced by the relative speed and sharpness of action in the acute blood infections of mice and rats and by the potency and duration of action in the subacute and chronic tissue infections of guinea pigs and rabbits. The accomplishment of a permanent cure was obtained in the experimental infections produced by five strains of pathogenic trypanosomes. *Tr. brucei*, *Tr. gambiense*, *Tr.*

evansi, *Tr. equiperdum* and *Tr. equinum*. Comparative experiments in laboratory animals with drugs which had been previously used, such as atoxyl, arsacetin, arsenophenylglycine, and the salvarsan derivatives, all of which had been previously used in the treatment of human trypanosomiasis, showed that tryparsamide was in many respects superior."

In her clinical observations made in the Belgian Congo several years ago, Doctor Pearce,³⁴ was able to substantiate the favorable indications which the experimental results with tryparsamide had given. It was shown that the drug was well tolerated and gives rise to no untoward symptoms except an occasional tendency, following too intensive a use of the drug, to produce visual disturbance in certain advanced cases. This, however, was usually transitory. The therapeutic results obtained in the preliminary use of the drug in 77 cases of sleeping sickness produced by *Trypanosoma gambiense* were very encouraging. More recently, Doctor Chesterman³⁵ in the Belgian Congo has definitely confirmed Doctor Pearce's general observations. From observations on the intravenous use of the drug he concludes that the maximum tolerated dose (which he believes should not exceed 4 grams per week for the full-sized adult) if given regularly for a period of about eight weeks is capable of completely removing trypanosomes from and rendering normal, the cell content of the cerebrospinal fluid of even the most advanced cases. This change is accompanied by a very marked clinical improvement which was observed to persist for practically a year, which was the longest time which had elapsed since his last use of the drug. Improvement was hardly less marked in cases which had resisted previous treatment with the drugs such as atoxyl. By a careful check on the patient it was possible to avoid the danger of any appreciable degree of visual disturbance. Up to the present, we see no reason to change our estimates of the value of the drug.

Doctor Smillie³⁶ has recently had the opportunity to extend the studies with tryparsamide in another direction, to the treatment of mal de caderas which has become one of the biggest eco-

onomic problems of the vast Paraguay Valley in South America, since this disease is causing the yearly loss of thousands of horses. In a preliminary communication he reports that the drug has been found to be definitely efficacious in the treatment of the early stages of the disease when the blood of the horse or mule is swarming with trypanosomes. This stage is from all practical considerations of the greatest importance to control.

But to return to human trypanosomiasis, it is seen that the two main directions which chemical efforts have taken to produce remedies for this disease have almost simultaneously yielded results which now give a more encouraging outlook with regard to its control—on the one hand, Bayer-205, most probably closely related to the benzidine dye group, and tryparsamide, a pentavalent arsenic compound. At the present time, it is perhaps too early to compare the relative efficacy of Bayer-205 and tryparsamide. There have been reports already with regard to Bayer-205 which indicate that the drug, although of definite value as a prophylactic, or in the early stages of the disease, has failed to give results in the more advanced cases. Tryparsamide, on the other hand, as Pearce and Chesterman have shown, has been the cause of definite improvement in patients even in the more advanced stages of the disease. Bayer-205 has been reported to exhibit a definite toxic behavior from which the patients or animals only slowly recover. This takes the form occasionally of an albuminuria. A general tonic effect has characterized the behavior of tryparsamide and perhaps the only untoward action has been the occasional visual disturbance which has been noted to follow too intensive a use of the drug. But, as before stated, this is in most cases of a temporary character. We shall perhaps have to wait a number of years before these drugs will have reached their final evaluation.

There is a temptation perhaps to speculate with regard to the chance that the more successful of the arsenical remedies which have been used for the treatment of human trypanosomiasis have been pentavalent compounds. In the case of

tryparsamide, there seems every reason to attribute its efficacy, not only to its powerful stimulating action on the animal economy and on the animal resistance, but to its great tissue penetrability and to its affinity for the tissues of the central nervous system which are involved in the more advanced stages of the human disease. This property has been demonstrated again in the behavior of the drug in certain types of neurosyphilis. In neurosyphilis there is a distribution of organisms in the central nervous system which is, in a sense, comparable to that which occurs in trypanosomiasis. This has in the past suggested the possibility that perhaps even at the time when the etiological relationship of paresis to syphilis was merely suspected, drugs which have proved efficacious in the treatment of human trypanosomiasis might find a usefulness in the treatment of paresis. Years ago, atoxyl, for instance, was repeatedly tried in this connection, but in all these attempts, it has proved quite inadequate. It was, however, a natural thought to consider tryparsamide in this connection, although its treponemacidal properties are not very marked. Nevertheless, in experiments in rabbits it had been shown to possess a marked action on the experimental lesions produced by the spirochetes. This was evidenced by their complete resolution and healing even in the presence of actively motile spirochetes which seemed to show little or no tendency to cause a recurrence.

A few years ago, Doctors Lorenz, Loevenhart, Bleckwenn, and Hodges,³⁷ in order to test the applicability of tryparsamide in this connection undertook a series of observations on a group of patients afflicted with paresis. They have concluded that tryparsamide on intravenous injection is definitely efficacious in the treatment of early paresis and certain other forms of neurosyphilis. Later observations have essentially confirmed this. It is perhaps too early to make a more precise statement with regard to the clinical use in all of its phases of tryparsamide in this disease, but sufficient is already known to demonstrate that it should have a field of usefulness in certain conditions for which there has been, heretofore, no efficacious mode of treatment.

If time would permit, still other phases of the progress which has been made in the application of chemical agents to the specific treatment of infectious diseases might be reviewed. But sufficient has been brought before you tonight to show that the results achieved have been far in excess of what was needed to justify the initiation of this type of investigation. In no sense, however, can any result such as the arsphenamines, Bayer-205 or tryparsamide be considered the summit of possible attainment and there is every reason to expect the ultimate discovery of more powerful agents. And as time goes on, new groups of substances and an ever-widening field for their therapeutic application may well be expected. There is perhaps no more hopeful direction for the continuation of effort. But those who engage in such undertaking must carry with them a certain measure of opportunism, since in spite of the constantly increasing number of positive observations, there is still lacking a general theory as to the chemical and physical factors which underlie specific therapeutic action. In this respect we are perhaps less informed than in the case of certain rules which seem to govern pharmacodynamic action. But I think that it is in this direction that further effort is required. We should not alone strive for the attainment of practical results, but continued attempts should be made to ascertain all of the theoretical factors which may contribute to chemotherapeutic action.

BIBLIOGRAPHY

- ¹ Koch, R.: Mitt. a. d. Kais. Gesundheitsamt., Bd. i, 234 (1881).
- ² Citation from Morgenroth, J., Therap. Monatshefte, xxvi, 97 (1912).
- ³ Bechhold and Ehrlich, P.: Zeitschr. f. physiol. Chem., xlvii, 173 (1906).
Bechhold: Zeitschr. f. physiol. Chem., lii, 177 (1907).
Bechhold: Zeitschr. f. Hygiene, lxiv, 113 (1909).
- ⁴ Jacobs, W. A.: Jour. Exp. Med., xxiii, 563 (1916).
Jacobs, W. A., Heidelberger, M., and Amoss, H. L.: Jour. Exp. Med., xxiii, 569 (1916).
Jacobs, W. A., Heidelberger, M., and Bull, C. G.: Jour. Exp. Med., xxiii, 577 (1916).
- ⁵ Browning, C. H., Cohen, and Gulbrandsen, R.: Brit. Med. Jour., i, 514 (1922).

- ⁶ Felton, L., and Dougherty, K.: Jour. Exp. Med., xxxv, 761 (1922) and unpublished observations.
- ⁷ Browning, C. H., Gulbransen, R., Kennaway, E. L., and Thornton, L. H. D.: Brit. Med. Jour., i, 73 (1917).
- ⁸ Felton, L. D., and Dougherty, K. M.: Jour. Exp. Med., xxxvi, 163 (1922) and unpublished observations.
- ⁹ Moore, H. F., and Chesney, A. M.: Arch. Int. Med., xix, 611 (1917); xxi, 659 (1918).
- ¹⁰ Browning, C. H., and Gulbransen, R.: Proc. Roy. Soc. B. xc, 136 (1917).
- ¹¹ Neufeld, F.: Zeitschr. f. Hyg., xxxiv, 454 (1900).
Schilling: Centr. f. Bakter., Orig. xxxi, 452 (1902).
Neufeld, F., and v. Prowazek: Arbeit, a. d. Kais. Gesundheitsamt, xxv, 494 (1907).
- ¹² Morgenroth, J., and Halberstädter, L.: Berl. klin. Wochenschr., xlvii, 646 (1910); xlviii, 558 (1911).
- ¹³ Morgenroth, J., and Levy, R.: Berl. klin. Wochenschr., xlviii, 1560, 1979 (1911).
- ¹⁴ Wright, A.: Lancet, ii, 1633, 1701 (1912).
- ¹⁵ Morgenroth, J., and Tugenreich, J.: Berl. klin. Wochenschr., liii, 794 (1916).
Morgenroth, J., and Tugenreich, J.: Biochem. Zeitschr., lxxix, 257 (1917).
- ¹⁶ Neufeld, F., and Engwer: Berl. klin. Wochenschr., xlix, 2381 (1912).
- ¹⁷ Moore, H. F.: Jour. Exp. Med., xxii, 269, 389 (1915).
- ¹⁸ Jacobs, W. A., and Heidelberger, M.: Jour. Am. Chem. Soc., xli, 2090 (1919).
- ¹⁹ Jacobs, W. A., and Heidelberger, M.: Jour. Am. Chem. Soc., xli, 2131 (1919).
- ²⁰ Jacobs, W. A., and Heidelberger, M.: Jour. Am. Chem. Soc., xli, 817; xlii, 1481, 1489, 2278; xlv, 1073, 1079, 1091, 1098.
- ²¹ Laveran, A., and Mesnil, F.: Ann. d. l'Inst. Pasteur, xvi, 1 (1902).
- ²² Ehrlich, P., and Shiga: Berl. klin. Wochenschr., xli, 329, 362 (1904).
- ²³ Mesnil, F., and Nicolle, M.: Ann. d. l'Inst. Pasteur, xx, 417, 513 (1906).
- ²⁴ Dale, H. H.: Physiol. Reviews, iii, 369 (1923).
- ²⁵ Händel and Joetten: Berl. klin. Wochenschr., lvii, 821 (1920).
- ²⁶ Mayer and Zeiss: Arch. f. Schiffs. u. Tropen-Hyg., xxiv, 257 (1920).
- ²⁷ Thomas, H. W.: Brit. Med. Jour., i, 1140 (1905).
- ²⁸ Ehrlich, P., and Bertheim, A.: Ber. d. deut. chem. Gesell., xl, 3292 (1907).
- ²⁹ Ehrlich, P.: Ber. d. deut. chem. Gesell., xlii, 17 (1909).
- ³⁰ Ehrlich-Hata: Die Exper. Chemotherapie d. Spirillosen., Berlin (1910).
- ³¹ Röhl, W.: Zeit. f. Immunitätsforsch., ii, 1, 496 (1909).
Berl. klin. Wochenschr., xlvi, 494 (1909).

- ³² Jacobs, W. A., and Heidelberger, M.: Jour. Am. Chem. Soc., xli, 1581, 1587, 1600, 1610, 1809, 1822, 1826, 1834 (1919); xliii, 1632, 1646 (1921).
- ³³ Jacobs, W. A., and Heidelberger, M.: Jour. Exp. Med., xxx, 411 (1919).
Brown, W. H., and Pearce, L.: Jour. Exp. Med., xxx, 417-96 (1919).
- ³⁴ Pearce, L.: Jour. Exp. Med., xxxiv, Supplement No. 1 (1921).
- ³⁵ Chesterman, C. C.: Trans Roy, Soc. Trop. Med. and Hyg., xvi, 394 (1923).
- ³⁶ Smillie, W. G.: Jour. Am. Vet. M. A., September, 1923.
- ³⁷ Lorenz, W. F., Loevenhart, A. S., Bleckwenn, W. J., and Hodges, F. J.: Jour. Am. Med. Assn., 80, 1497 (1923).

ETIOLOGY AND PREVENTION OF SIMPLE GOITER *

DR. DAVID MARINE

Director of Laboratories, Montefiore Hospital, New York

INTRODUCTION

SIMPLE or endemic goiter fortunately is of little practical importance to a local audience although as regards world-medicine it is one of the most important and wide spread causes of human suffering and of physical and mental degeneracy with which society has had and still has to deal. The frequency of simple goiter in the northern portion of this state has aroused public opinion to the importance of its control and prevention.

If the thyroid were guilty of all the ills it has been accused of, and performed all the functions that have been ascribed to it, it would, without doubt, be at the same time, one of our greatest malefactors as well as one of our greatest benefactors.

I am one of those who believe that the thyroid has very few functions to perform as organ functions go, and that we are now in a general way familiar with the most important of these functions. In other words, we probably possess more definite knowledge concerning the anatomy, physiology, chemistry and pathology of the thyroid than of any other gland. On the other hand, as regards the interrelations of the thyroid function with the function of other organs, we are only at the beginning of definite information. Enough is known to recognize that a vast and almost unexplored field of great physiological importance is involved.

Broadly defined, our present conception of the function of the thyroid is that it provides the means through its iodine containing hormone of maintaining a higher level of metabolism than would otherwise obtain and also through fluctuations in

* Delivered January 19, 1924.

its activity it provides a means for varying the rate of metabolism to meet changing physiological needs.^{1, 2}

On the basis of this conception of the normal function of the thyroid, the diseases associated with disturbances of its function may be divided into two groups, as follows:

I. Thyroid Insufficiencies

(1) Simple goiter (endemic, epidemic, sporadic)

(2) Myxedema

(a) Infantile (cretinism)

(b) Adult (Gull's disease)

II. Exophthalmic goiter.

Reduced to more popular terms these groups would be designated Hypo- and Hyperthyroidism. They overlap to some extent. Myxedema may supervene in exophthalmic goiter and exophthalmic goiter may develop on the basis of simple goiter although there does not appear to be any necessary relationship between the two groups.

Thyroid Insufficiencies.—From the standpoint of clinical medicine, simple goiter and myxedema are usually treated separately, but from the standpoint of physiology and pathology they are but different stages or degrees of the same nutritional fault. So far as is known, Paracelsus³ was the first to insist on the close relationship between goiter and cretinism. All subsequent study has strengthened this association which has perhaps been most clearly and briefly expressed by Morel,⁴ in 1864, as follows: "Goiter is the first stage on the road to cretinism." The same idea has been more comprehensively expressed by Koestl⁵ as follows. "The condition which produces goiter when it is weak also produces cretinism when it is more intense."

The publication of Gull's⁶ classical paper in 1874, entitled, "On a cretinoid state supervening in adult life of women," with which he definitely associated atrophy of the thyroid, followed by that of Kocher,⁷ in 1883, in which he reported 16 cases of complete thyroidectomy in humans which developed a cachexia similar to that described by Gull, to use Kocher's words, left no doubt of an unmistakable relationship between myxedema

and cachexia strumpriva on the one hand, and cretinism on the other. The final proof of the relation of the thyroid to myxedema was added in 1891 when Murray⁸ cured a case by administering a glycerol extract of sheep's thyroid.

What follows will deal particularly with simple goiter although on account of the association just mentioned infantile myxedema (cretinism) must come into the discussion.

Occurrence and Distribution.—Simple goiter includes those thyroid enlargements in man and animals which were formerly grouped under endemic, epidemic, sporadic and physiologic. It may occur in any land and fresh-water animal with the ductless thyroid. Animals living in the sea are free from the disease. On the sea coast, generally, it is ordinarily rare in man, and the cases seen are usually in women and in association with pregnancy and lactation. It occurs in all races, in all climates and at all habitable altitudes. In the temperate zones there is a seasonable variation, both in man and animals. It develops more frequently during the late winter and early spring months. Similar seasonal variations also occur in the iodine store of the thyroid as pointed out by Seidel and Fenger.⁹ While goiter may occur anywhere, even in mid-ocean, as on one of Captain Cook's voyages in 1772, one of the most striking characteristics is the increased incidence in certain more or less defined regions of the world, the so-called districts of endemic goiter, (Hirsch¹⁰). The most notable of these districts are the Himalaya Mountain region of South Central Asia, the Alps, Pyrenees and Carpathian Mountain regions in Europe, the Andean plateau of South America. In North America the most important areas are the St. Lawrence River and Great Lakes basin, extending west through Minnesota, the Dakotas and the adjacent Canadian provinces and also the Pacific Northwest, including Oregon, Washington and British Columbia. Less important foci occur throughout the Appalachian Mountain region, the Rocky Mountain states and states in the Great Central Basin. It will be noted that most of these regions are mountainous, although there are numerous exceptions. Of greater importance is the occurrence of endemic goiter for the



(Courtesy of the U. S. Geological Survey.)

FIG. 1.—Illustrates the southern border of the silt deposit and of the ice sheet of the glacial epoch. The areas of endemic goiter correspond closely to the areas covered with ice or with glacial silt.

most part on soils deposited from the last glacial period. (See Fig. 1.)

There is also general evidence of variations in the incidence of goiter in these districts during the last 100 years. There are many reports of the sudden occurrence of large numbers of goiter, the so-called epidemics, both in man and animals. In man these so-called epidemics have for the most part occurred in military garrisons, in institutions and in schools.^{11, 12} These outbreaks have usually been in districts where the ordinary incidence of goiter is high and in new inhabitants. I have had opportunity of investigating such outbreaks in dairy herds, in poultry farms and in fish hatcheries.¹³ Some of these outbreaks were in goiter regions while others were not, showing that with the optimum conditions for its development present, goiter may occur anywhere.

Beginning about the age of puberty, females are more often affected than males. In the districts of severest endemic goiter this difference in incidence due to sex is masked.¹⁴ In such regions all of the inhabitants may be affected. In non-goiter regions where only sporadic cases occur these are usually seen in women. Striking an average between these two extremes one may say that in general, simple goiter is two or three times more common in females. In the lower animals a difference in incidence due to sex has not been demonstrated. The periods in life when simple goiter most frequently develops are (1) during fetal life, (2) during pregnancy and lactation, (3) during puberty and (4) during menopause.

Etiology.—The cause of thyroid hypertrophy and hyperplasia has interested medical men from the earliest days of medical history. There are few, if any diseases in which such a variety of agents have been brought forward as causal factors. St. Lager, in 1867,¹⁵ enumerated over 40 theories concerning its causation. Many of these theories must now be grouped as folklore and legendary in nature. Poverty, unhygienic living conditions and surroundings, unbalanced diets, certainly are important indirect contributing causes. McCarrison¹⁶ states that it is a common saying in certain provinces of Northern India

that people who can afford the luxury of table salt are less likely to be affected. A great variety of organic and inorganic substances, both of known and unknown chemical nature, occurring in soil and water have been suspected as causal factors. Among these may be mentioned arsenic, salts of calcium and magnesium, sulphides, particularly of iron, fluorides, silica and miasms from decomposing vegetable matter. These substances are not now believed to play any direct role. With the development of bacteriology and protozoology the view that thyroid enlargement might be due to a specific living virus was widely adopted. This view has been the basis of extensive investigation. Many types of parasites, bacteria, fungi and protozoa, have been described. McCarrison¹⁷ has worked extensively in this field in India and formerly believed an organism of the colon group was responsible. Infection, he believed, took place through drinking water and food. Chagas¹⁸ working in Brazil in 1909, reported a form of goiter which he thought was due to a trypanosome (*Schizotrypanum cruzi*). Subsequent work has shown that the infection with this organism may exist without thyroid enlargement and that thyroid enlargement may exist without the infection. Kolle,¹⁹ of the Swiss Goiter Commission, failed to produce thyroid hyperplasia in rats by inoculating them with cultures of this organism. The occurrence of this infection in a district of endemic goiter readily explains the association.

Numerous experiments where fresh and boiled water from supposedly goiter-producing springs were employed have been carried out in recent years by Dieterle, Hirschfeld and Klinger,²⁰ by Bircher,²¹ by Wilms²² and by the Swiss Goiter Commission,²³ These experiments have yielded either negative or doubtful results. In general, nothing suggesting a direct infecting agent has been proven, despite the enormous amount of work which has been done. That toxins of bacterial and other origins may indirectly stimulate the thyroid to increased activity and possible enlargement is well known. Thyroid enlargements are frequently seen in early pulmonary tuberculosis, in active syphilis, in acute fevers like pneumonia, scarlet fever and diphtheria. The

thyroid reaction in these conditions is now explained as dependent upon its increased function in febrile diseases which in turn brings about the increased metabolism occurring in infections and indicates that the thyroid is an element in the defensive mechanism against infection and intoxication.

Water has been associated as the carrier of the active goiter-producing agent by all peoples from the remotest times.²⁴ Livingston has reported that the inhabitants of Central Africa held this belief. Barton²⁵ stated that the American Indians living in the region of the Great Lakes also believed that water conveyed the causal agent and it is highly improbable that these peoples were familiar with similar views held by the Chinese, the Greeks and the Romans. The conflicting experimental data thus far reported could perhaps be better interpreted as pointing to the lack of some substance (iodin?) necessary for the prevention of thyroid enlargement than that it contained some virus or toxin capable of producing thyroid hyperplasia. My own interest in water as a carrier of the goiterogenic agent ceased in 1911 when (1) Lenhart and Marine²⁶ demonstrated that brook trout living in wooden runways (about 30 feet long) between ponds recovered from thyroid hyperplasia although all the trout living in the ponds above and below the runways were highly goiterous and (2) when it was demonstrated that fish with active thyroid hyperplasia put in the tail race below all ponds regularly recovered although living in the most polluted water. If water is a factor in thyroid hyperplasia (and I believe it is) it is due to the absence rather than the presence of some substance. Many years ago²⁷ we demonstrated that certain species of fish (*Esox*) living in Lake Erie had enlarged thyroids, just as did the land animals of the adjacent country.

Lastly, we will take up the present view of the etiology of simple goiter. This view assumes that goiter is a work or compensatory hypertrophy of the thyroid depending upon a relative or absolute deficiency of iodine. On this basis goiter is only a symptom of a specific deficiency disease in which an insufficient supply of iodine for one or another cause is the chief factor. The idea that goiter is due to a lack of iodine is not new.

Prevost²⁸ (1830), Maffoni,²⁸ Inglis²⁸ (1838), Marchand,²⁸ Niepce,²⁸ and particularly Chatin,²⁹ who published the results of his researches in 1852, claimed to have demonstrated a lack of iodine in the air, soil and water in districts of endemic goiter. His claims were attacked, and unfortunately so discredited on the grounds of faulty and inadequate methods that further study of this important subject was blocked for forty-five years.

It was not until 1895 when Baumann,³⁰ of Freiburg discovered that iodine in a firm organic combination was a normal constituent of the thyroid, that the possible importance of iodine in thyroid function was revived. He did not succeed in showing that iodine was a necessary constituent although he and his pupils, Roos and Goldman,^{31, 32} were able to show that feeding iodine or foods rich in iodine caused a storage of this element in the gland and that in general goiterous thyroids contained less iodine than normals. Oswald,^{33, 34, 35} in 1899, showed that the iodine compound was contained in the globulin or colloid of the gland and that glands rich in colloid usually, though not always, contain more iodine than hyperplastic glands. Further investigations of the nature of this iodine-containing compound resulted in its isolation in 1914 by Kendall³⁶ in crystalline form and named by him thyroxine. This substance has been discussed before this Society by its discoverer.

We may now review in more detail some of the relations which iodine bears to thyroid morphology and function. The relation of iodine to the structure of the thyroid in normal glands, in developing and involuting hyperplasia was first extensively investigated by Marine and Williams,³⁷ and by Marine and Lenhart.^{38, 39, 40} These studies established the foundation on which the later experimental proof of the fundamental importance of iodine in thyroid physiology and pathology was developed. In comparing the microscopic appearance with the iodine contents of several hundred human and canine goiterous and non-goiterous thyroids, a striking relationship between the iodine store and the histological structure was made out. (See Figs. 2 and 3.) In the thyroids with normal structure the iodine contents varied between 5.5 mg. and 1 mg. per gm. of dried gland.

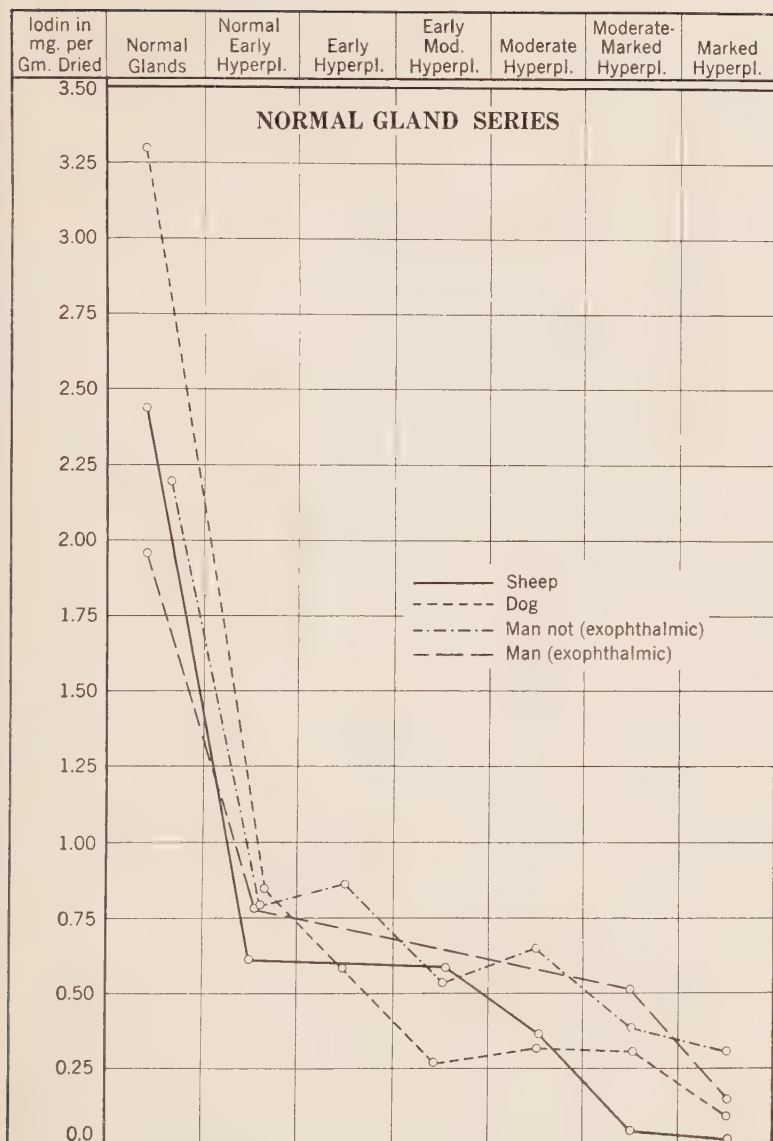


FIG. 2.—Illustrates the relation of the iodine store in sheep, dog and human thyroids to their histological structure. (Normal thyroid series). (From The Archives of Internal Medicine, 1909, 4, 440, by courtesy of the Editors.)

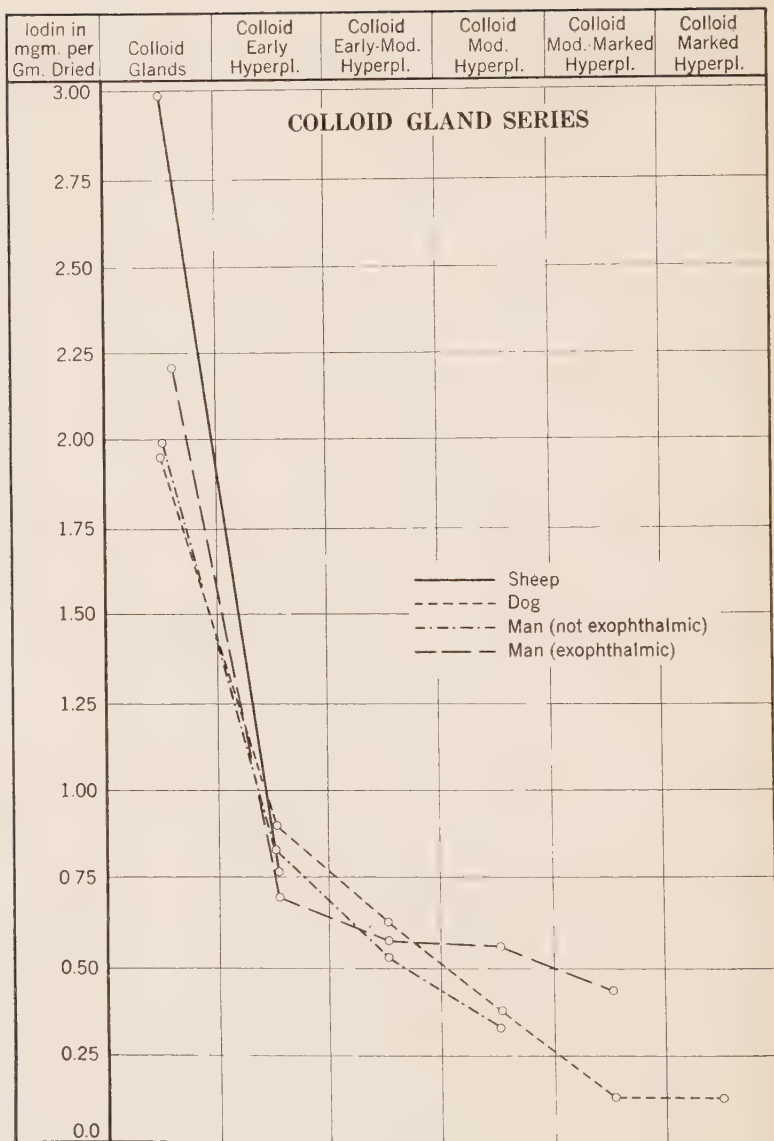


FIG. 3.—Illustrates the relation of the iodine store in sheep, dog and human thyroids to their histological structure. (Colloid gland series). (From The Archives of Internal Medicine, 1909, 4, 440, by courtesy of the Editors.)

When the iodine store was found below 0.1 per cent. hypertrophic and hyperplastic changes were regularly found. Comparing further the varying degrees of thyroid hyperplasia with the iodine store of such glands it was found that the iodine store progressively decreased as the degree of hyperplasia increased so that in the most marked hyperplasias iodine was absent or present only in traces. A similar relationship between the iodine store and histological structure of the gland has been observed in large series of ox, pig, sheep, rabbit and fowl thyroids. The generalization may, therefore, be made that the iodine store varies inversely with the degree of active hyperplasia and that if the iodine store is higher than 0.1 per cent. of dried weight in these animals no hyperplastic changes are present. This generalization could be readily tested experimentally in several ways:

In the first series of experiments iodine was fed to dogs with hyperplastic thyroids after a small piece of the gland had been removed for iodine determination and anatomical classification. Without exception there was found a remarkable storage of iodine in the glands associated with an involution of the hyperplasia to the colloid or resting state.^{41, 42, 43}

Similar observations have been carried out on rabbits, cats, sheep, fowl and fish. Complete involution of a marked hyperplasia requires about fifteen days in dogs and about forty days in brook trout.⁴⁴ Sweeping statements in medicine are dangerous, but in this instance, after many thousand observations, I believe it can be stated that iodine will invariably bring about involution physiological over-growth of the thyroid. The growth of thyroid carcinoma in both men and dogs is not affected by the administration of iodine nor is iodine retained in active thyroid carcinoma tissue even after many months of feeding.⁴⁵ The normal thyroid cell has an extraordinary affinity for iodine. This may be demonstrated in both *in vitro* and *in vivo* experiments.^{46, 47} As much as 18.5 per cent. of a single dose of 38 mg. of iodine given as potassium iodide to a dog by mouth has been recovered from its thyroid whose ratio to body-weight was as 1 to 687. Perfused surviving thyroid shows the same marked ability to take out and store iodine from the circulating fluid as is seen in the intact

gland. Similar perfusions of the kidney and spleen do not result in the retention of iodine (Marine and Feiss).

In a *second* series of experiments Marine and Lenhart⁴³ studied the effects of the administration and withholding of iodine after partial removal of the thyroid in dogs. As has been shown by many observers, notably by Halsted,⁴⁹ if one removes from one-half to three-quarters of the gland the remainder undergoes compensatory hyperplasia histologically identical with that seen in the spontaneous hyperplasia of developing goiter.

Marine and Lenhart have carried out large numbers of experiments of partial removal in dogs where the histological condition and iodine store were compared and it was found that compensatory hyperplasia did not begin until the iodine store in the remaining part had fallen below 0.1 per cent., and that the degree of compensatory hyperplasia increased proportionately as the iodine store decreased just as was found in the spontaneous hyperplasias. On the other hand, when we removed as much as three-fourths of the gland and administered traces of iodine, no compensatory hyperplasia took place in the remaining portions which, as pointed out above, would regularly occur if iodine were withheld. Iodine in any amount will not protect against compensatory hyperplasia when much more than three-fourths of the gland is removed, although the administration of the preformed iodine containing hormone as desiccated thyroid will still further protect the remaining portion against hyperplasia.

A similar effect of iodine may be demonstrated in thyroid transplants in rabbits. As has long been known, thyroid autografts readily in any part of the body. Manley and Marine⁴⁸ have shown that iodine will prevent the growth and hyperplasia occurring in such transplants or bring about an involution of the hyperplasia if it has occurred irrespective of the location of the transplanted tissue, just as it prevents or involutes compensatory hyperplasias occurring in non-transplanted tissues. Such transplants also are capable of storing iodine.

These simple experiments show clearly that the changes in the iodine store and histological structure occurring in experi-

mentally controlled hyperplasias are identical with those observed in spontaneous hyperplasias or simple goiter.

The same general reaction of the thyroid can also be produced in experimental congenital goiter. Halsted ⁴⁹ first experimentally produced congenital goiter in dogs in 1889. He observed that puppies born from mothers which had had most of the thyroid gland removed had enlarged actively hyperplastic glands at birth—the enlargement reaching twenty times the normal size. He also noted that the histological appearance of such congenital thyroid hyperplasias was identical with the compensatory hyperplasias following partial removal of the gland. The fact that removal of most of the thyroid from the mother ordinarily causes marked enlargement of the fetal thyroid has been confirmed from many sources and for many animals (Edmunds,⁵⁰ Marine and Lenhart,⁴³ Carlson ⁵¹). In addition to confirming Halsted's observations on dogs, Marine and Lenhart,⁴³ in 1909, added the additional fact that if a few milligrams of iodine are given during pregnancy to dogs from which most of the thyroid has been removed the young at birth will have normal thyroids, both as regards weight, histological structure and iodine content. We have also been able to obtain from the same animal alternate litters of goiterous and non-goiterous puppies by withholding and administering iodine. These experimental congenital thyroid hyperplasias are true congenital goiters. They are identical with the spontaneous congenital goiters of man and animals and are dependent upon a maternal functional insufficiency of the thyroid. As in the case of many facts of medicine, the experimental production of congenital goiter was merely laboratory confirmation of the views held by the older students concerning the etiology of congenital goiter in man. The experimental production and control of congenital goiter should end the discussion in support of the true hereditary nature of congenital goiter.

Another series of experiments was carried out to demonstrate that the normal thyroid could be kept normal over long periods of time even in highly goiterous districts by the administration of small amounts of iodine. In these experiments litters of puppies from iodized mothers were used to insure normal

thyroids at birth. To half of each litter one milligram of iodine was given by mouth each week, while the other half of each litter served as controls. These experiments were carried out in Cleveland, where practically all dogs normally have some degree of thyroid hyperplasia. It was found that those puppies which did not receive iodine developed the usual thyroid enlargement while the thyroids of those that received a milligram once a week remained normal, although both control and iodine fed puppies were kept in the same kennels, and fed with the same kind of food. These experiments proved that iodine, in minute amounts, could control thyroid over-growth and that we had to deal with a unique physiological fact in that a single inorganic element determined the functional value of the thyroid secretion.

Diet plays a part in the etiology of thyroid over-growth (goiter).⁵² St. Lager¹⁵ (1867) stated that foods rich in fats—particularly pork had long been considered as an important factor in the cause of goiter. Baumann and his pupils noted that foods rich in iodine (codfish) caused a storage of iodine in the gland and that fresh meats caused a decrease in the iodine store. Watson⁵³ also found that meat diets caused hypertrophy and hyperplasia of the rat's thyroid. Marine and Lenhart showed that pig's liver was the most potent of a great variety of meats in causing thyroid hyperplasia in dogs. We also found that this food was an important factor in the causation of thyroid overgrowth in brook trout, a disease which at that time (1910), was widespread throughout the fish hatcheries in the United States where the Salmonidæ were artificially reared.^{13, 26}

In investigating an acute outbreak of goiter in poultry in the spring of 1914, the high protein and fat content (meat scrap) of the diet was believed to be an important factor in causing thyroid overgrowth. We also noted in a herd of dairy cattle in which several goiterous and cretin calves were born that the high proportion of cotton-seed meal used in the food was probably an important factor. McCarrison⁵⁴ has shown that diets which include in excessive amount of digestible fats free from iodine (lard and butter) were even more potent in producing

thyroid hyperplasia in pigeons than were the high protein and fat diets above mentioned. These observations on the effects of fat feeding have been confirmed by Mellanby.⁵⁵ McCarrison further showed that fats rich in iodine, as codliver oil, did not cause thyroid hyperplasia. This probably explains why goiter is rare among the Eskimos in spite of their high fat and protein diets. Shapiro and Marine⁵⁶ (unpublished) confirmed McCarrison's observation by showing that the administration of 10 c.c. of cotton-seed oil daily to rabbits in addition to their ordinary diet regularly causes as marked thyroid overgrowth in rabbits as the diet of pig's liver did in brook trout. It usually takes from 60 to 90 days to produce marked thyroid enlargements in rabbits. As thyroid hyperplasia is secondary to the depletion of the iodine store these facts indicate that diet rich in proteins and fats increase the rate of discharge of iodine. It seems probable that thyroid activity is more necessary for the metabolism of fats and proteins than of carbohydrates. Carbohydrate diets as shown by McCarrison do not cause thyroid hyperplasia.

Lastly, I will call attention to the influence of *pregnancy and lactation* in the etiology of thyroid enlargement. The period of pregnancy and lactation is one of the three important periods in life when simple goiter most frequently develops. The association has been known since antiquity. Lawson Tait,⁵⁷ in 1875, described the step like enlargement of the thyroid associated with multiple pregnancies. Slight hypertrophy of the thyroid during pregnancy often to the stage of clinical detectability is looked upon by obstetricians as normal even in non-goiterous districts. The physiological nature of the enlargement has not been extensively investigated. Naturally one would suspect from what we know of normal thyroid physiology that the hypertrophy during pregnancy was dependent upon increased demands for functional activity during this period. Murlin,⁵⁸ in his studies on metabolism during pregnancy, both in human cases and in dogs, observed only a slight increase in the total energy production, averaging about 4 per cent. in the case of women, and 9 per cent. in a dog which was proportional to the increase in

the weight of the fetus. Baer,⁵⁹ in forty-five normal human cases, found the basal metabolic rate in late pregnancy to average around 30 per cent. above the normal for non-pregnant women, of approximately similar surface areas. Root and Root⁶⁰ in a carefully studied human pregnancy found an increase beginning at the fifth month and reaching +23 per cent. eleven days before delivery. During the last two years, we have studied the influence of pregnancy in rabbits, with and without thyroidectomy, on heat production.⁶¹ Our results show that heat production markedly increases in this animal during pregnancy and lactation. The rise begins about the middle of pregnancy, that is about the end of the second week of gestation, and continues through the period of lactation. Thyroidectomy performed during the first week of pregnancy greatly reduces and sometimes prevents the increased heat production of both pregnancy and lactation. Thyroidectomized rabbits occasionally abort, but we have never seen an instance of abnormally prolonged pregnancy after thyroidectomy as has been described. A large percentage of these rabbits abandon their young at birth. These experiments we believe indicate that normally there is a greater increase in heat production during pregnancy than some of the previous work has indicated, and that this increase is in part dependent upon the maternal thyroid and that the enlargements which may occur at this time are essentially compensatory or work hypertrophies. The enlargement of the fetal thyroid, which is so directly dependent upon maternal thyroid insufficiency is also, we believe, a purely physiological or compensatory reaction.

To sum up the evidence at present available concerning the etiology of goiter, I believe we may abandon the older views that it is due to a specific living virus or a specific chemical substance and conclude that simple goiter is a compensatory or work-hypertrophy depending upon a variety of metabolic stimuli for functional activity of the thyroid which depletes its iodine store. The immediate cause of thyroid enlargement is a relative or an absolute deficiency of iodine. This deficiency may result from:

(1) Any factors which increase the needs of the organism for the iodine-containing hormone as occur during puberty, pregnancy and lactation, during the menopause, during certain infections and intoxications, following sufficient injuries to the interrenal gland, or as a result of diets consisting mainly of fats and protein.

(2) Any factors which interfere with the absorption or utilization of the normal intake of iodine. We have no knowledge as yet of such factors, although it is conceivable that the intestinal bacterial flora or intestinal parasites could utilize or divert part of the iodine intake.

(3) Factors which bring about an abnormally low intake or actual deprivation of iodine, either natural or experimental. The normal source of iodine is from food and water though traces may be taken by breathing the air in the immediate vicinity of the sea. In districts of endemic goiter both the food and water derived from such soils have been proven to be very low in iodine.²⁹ (Olin,⁶² McClendon.⁶³)

When one recalls that the maximum store of iodine in the normal thyroid is about 25 mg., and that an intake of 50 mg., if properly distributed may maintain the thyroid in a normal state for as long as a year, that certain diets, pregnancy, fevers and other conditions bringing about increased metabolism can quickly exhaust the iodine store of the thyroid, it would appear that the iodine deficiency might be considered as a primary and possibly the essential cause of goiter. Nevertheless, we must still consider the possibility that some chemical agent or toxin may be operating in districts of endemic goiter to divert an otherwise adequate intake of iodine or to increase the needs of the organism for thyroid activity, and not rest content merely with the fact that for practical purposes thyroid hyperplasia is due to iodine deficiency.

Anatomical Cycle and Pathological Anatomy.—The thyroid is a very labile tissue capable of marked and rapid hyperplasia and involution in response to variations in functional activity. On this account a wide range and a great variety of progressive, regressive, degenerative, inflammatory, atrophic and neoplastic

of active hyperplasia and such colloid glands are capable of manifesting all of the reactions of which a normal gland is capable.⁴² Thus, they can repeat the cycle of hypertrophy, hyperplasia and involution many times during the life-history of the animal. The step-like enlargements seen in association with multiple pregnancies is a clinical manifestation of alternating hyperplasia and involution. This has been shown experimentally in dogs by producing compensatory hyperplasia by partial removal of the gland, then involuting this hyperplasia with iodine; again producing compensatory hyperplasia by partial removal and again involuting with iodine. We have succeeded by this means in causing the gland to repeat its cell cycle as many as seven times in the same animal during a period of eighteen months.

These cell changes are not specific for any clinical disease^{66, 67} as some have supposed, but occur in response to any stimulus for increased thyroid activity which brings about a sufficient depletion of the iodine store.

The first change in the thyroid in developing goiter is a marked decrease in the iodine store. When this has fallen below 0.1 per cent. per gm. of dried gland increased vascularity, cell-hypertrophy and hyperplasia occur. The stainable colloid decreases as the hyperplasia increases. In the lower animals, with the exception of the rat, this hyperplasia of the epithelium is regular and uniform, while in man it is frequently irregular and nodular—so-called struma nodosa or adenomatous goiter.⁶⁸ These nodules or adenomas are due to different rates of growth of foci of thyroid cells in response to a general stimulus to the thyroid. It is believed they may arise both from differentiated thyroid alveoli and from cell rests as first described by Wölffler.⁶⁹ These adenomas are an integral and essential part of endemic goiter in man and are due to the same stimulus which excites the thyroid as a whole to hypertrophy. These multiple circumscribed benign growths are functionally active, yet have certain of the attributes of tumor, one of which is that their growth once initiated is frequently not controlled by iodine as are all simple hyperplasias.⁷⁰ The terminal metamorphoses are far more serious than those of simple goiter. Since in addition to pressure effects,

hemorrhage, necrosis, cyst formation and their possible rôle in the etiology of Grave's disease, probably 90 per cent. of the malignant tumors of the thyroid arise from them.

Prevention.—The curative treatment of simple goiter and cretinism has been actively practised for some 2500 years without any notable decrease in its incidence from this source. Nor has the introduction of surgery during the last 50 years materially affected the general problem of control.

There is some general evidence that the severer degrees of goiter and cretinism are less common than formerly and also that certain regions where mild endemic goiter formerly existed are now relatively free. It is possible that this result is an indirect effect of the general economic betterment—higher standards of living, improved sanitation, better and larger varieties of food, control of infectious and contagious diseases and more extensive means of transportation. Some more direct method of prevention must be developed if we are to control the disease. The search for such methods is not new. This was one of the main purposes of the Goiter Commissions appointed by the Sardinian government in 1845, by the Austrian government in 1860, by the French government in 1863, by the Swiss government in 1908, and more recently by the Italian government. The installation of new water supplies was recommended by some of the goiter commissions. In certain instances this is said to have decreased and in others to have increased the incidence. In Vienna, Austria, Portland, Oregon, and Seattle the new water supplies, even though exceptionally low in bacteria, organic and inorganic matter seem to have resulted in an increase in goiter. Changing the water supply for the purpose of goiter prevention is at present without scientific support. As a result of the experimental data regarding the relation of iodine to thyroid physiology and pathology and the conclusion drawn from them, that simple goiter was due to a relative or absolute deficiency of iodine, the plan of supplying minute amounts of iodine to meet this deficiency suggested itself.

The first occasion where iodine was thus used in the prevention

of simple goiter on a large scale was in 1909 and 1910^{13, 26} when Marine and Lenhardt demonstrated that this substance added to water supply in a concentration not greater than 1 mg. per liter arrested and prevented the development of thyroid hyperplasia in brook trout. It was later shown that changing the diet of the fish to whole sea-fish, which also contained traces of iodine, was equally efficacious.⁷¹ Since then the administration of iodine in some form or manner has been successfully applied in the prevention of goiter in sheep, cattle, pigs and poultry.^{72, 73, 74} Attempts to apply the plan of goiter prevention in man on a large scale were beset with difficulties, obstacles and hostility which society seems always to have interposed when new methods of controlling diseases (however meritorious) have been proposed. Many interesting stories could be told of our efforts and failures to make the experiment.

The prevention of simple goiter in man was not attempted on any large practical scale until 1917⁷⁵ when Dr. O. P. Kimball and I began its practical application among the girls attending the public schools of the city of Akron, Ohio. The method used at that time consisted in the administration of 2 gms. of sodium iodide in 0.2 gm. doses distributed over a period of two weeks and repeated each autumn and spring. At that time it was pointed out that this amount of iodine was excessive and far beyond the needs of the individual or the ability of the thyroid to utilize or store it. This demonstration was carried out for a period of two and one-half years and the results are briefly as follows: Of 2190 pupils taking 2 gms. of sodium iodide twice yearly only five developed thyroid enlargement while of 2305 girls not taking the prophylactic, 495 developed thyroid enlargement. Of 1182 pupils with thyroid enlargement at the first examination who took the prophylactic, 773 thyroids have decreased in size; while of 1048 pupils with thyroid enlargement at the first examination who did not take the prophylactic, 145 thyroids have decreased in size.⁷⁶

These figures demonstrate in a striking manner both the preventive and the curative effects of minute doses of iodine.

They are just as striking as the results obtained in animal experiments and the generalization may be made that human goiter is as easily and as cheaply prevented as it is in the lower animals. Since these results were reported numerous papers have appeared in the European literature particularly from Switzerland and Italy, confirming these observations and extending the prophylactic use of iodine. Klinger⁷⁷ in 1921 reported even more striking curative results in the school children of Zürich. He worked with a school population in which the incidence of goiter varied from 82 to 95 per cent. while our maximum incidence in Akron was 56 per cent.

Thus, of 760 children, 90 per cent. were goiterous at the first examination. After 15 months' treatment with 10 to 15 mg. iodine weekly only 28.3 per cent. were goiterous of a total of 643 reexamined. Independent reports by Fritsche,⁷⁸ Bayard,⁷⁹ Hunziker and Wyss,⁸⁰ de Quervain⁸¹ from different Swiss towns indicate equally beneficial results. Similar reports from Italy have been made by Muggia⁸² and Pighini⁸³ during the last two years. In 1922 the Swiss Goiter Commission⁸⁴ recommended the introduction of state wide prophylactic measures against endemic goiter in either of two ways:

1. For general prophylaxis the use of salt containing 2 to 5 mg. iodine per Kg. and
2. For school prophylaxis the use of tablets containing 1 mg. iodine at weekly intervals.

These amounts of iodine are too small.

Iodine is effective when administered in any form or by any means:—by inhalation, by mouth, by application to the skin or by injection. This fact introduces difficulties and advantages—difficulties as regards the selection of the best form and means of administration, and advantages in that the desired results may be accomplished with certainty in a variety of ways. The ideal plan of administration is still to be worked out. Historically^{85, 86, 87} and of course quite unknowingly iodine containing salt was the first means of administration and I am of the opinion that ultimately it will prove the best means of adminis-

tration where the entire population is to be protected. Sea salt if used exclusively or a salt containing 2 to 5 mg. iodine per Kg. would seem ample in the milder districts of endemic goiter. If applied to the school population only, Swiss plan of giving tablets containing 5 to 10 mgm. at weekly intervals is more convenient and quite sufficient for prevention. For curative effects larger amounts will probably be necessary. Simple goiter most frequently develops (1) during fetal life, (2) during adolescence, and (3) during pregnancy and lactation. Any plan of prevention that controls thyroid growth during these three periods would practically eliminate simple goiter. Goiter in the mother and fetus can be prevented as easily and as simply as that of adolescence and by the same means. All pregnant women living in goiter districts should be given the equivalent of 10 mg. iodine each week during the period of pregnancy and lactation.

The dangers and untoward effects of iodine when used in the minimum amounts suggested have been exaggerated. Iodine like other foods has been greatly abused and its abuse in the prevention and treatment of goiter will continue until it is realized that the maximum storage capacity of the normal thyroid for iodine is not over 25 mg. and that such a store is sufficient to meet the ordinary physiological needs of the organism for months. When one considers the extent to which iodine is daily used, both by the profession and the public the probability of injury becomes negligible. Iodism and the possibility of aggravating incipient exophthalmic goiter are the only dangers worthy of consideration. In general iodine is too dangerous a drug to be used in Grave's disease although there are unquestionably stages of the disease in which daily doses of 1 mg. may be helpful.

Desiccated thyroid is theoretically a better prophylactic than iodine but practically it is too dangerous a drug to be recommended at present.

Cretinism.—Prevention offers the only certain means of controlling endemic cretinism both in man and in animals. In sharp contrast with Gull's disease postnatal treatment is only partially successful because too much damage has been done before treat-

ment can be instituted. Remarkable cures may be obtained in cretin animals when treatment can be started shortly after birth ⁸⁸ Since all available evidence indicates that endemic cretinism is due to the same nutritional fault as endemic goiter and since congenital myxedema in animals may be so easily controlled by the administration of iodine to the mother during pregnancy, I believe the prevention of endemic cretinism in man is as simple as the prevention of simple goiter and can be accomplished by the same means.

Simple goiter is the easiest and cheapest of all known diseases to prevent and its control may be accomplished by available methods as soon as organized society determines to make the effort. The prevention of goiter will mean a great deal more than eliminating this form of cervical deformity. It means in addition the control of those forms of physical and mental degeneracy: such as cretinism, mutism and idiocy, which are dependent upon thyroid insufficiency. Further, it would prevent the development of thyroid adenomas. The terminal metamorphoses of adenomas are far more serious than those of simple hyperplasia since in addition to hemorrhage and cyst formation probably 90 per cent. of the malignant tumors of the thyroid arise from these growths. Many investigators have likened the effects of thyroidectomy and the manifestations of cretinism to premature senility. Arteriosclerosis commonly occurs in thyroidectomized lambs (Simpson), ⁸⁹ in myxedema (of adults) and in endemic myxedema (cretinism). It has also been pointed out that carcinomas in general appear earlier in life in highly goiterous districts than in non-goiterous districts. The elimination of endemic goiter would eliminate this particular form of premature senility and the sequellæ which it entails.

In conclusion, one can say that the factors which cause simple goiter center about the supply of iodine and the needs, normal and abnormal, of the thyroid gland for iodine. Supplying this element in amounts that roughly could be considered as approximating the physiological demands has resulted in preventing

simple goiter in both man and animals and in preventing cretinism in animals.

BIBLIOGRAPHY

- ¹ Marine, D.: The present status of the functions of the thyroid gland. *Physiol. Reviews*, 1922, ii, 521.
- ² Magnus-Levy, A.: Untersuchungen zur Schilddrüsenfrage. *Zeitschr. f. klin. Med.*, 1897, xxxiii, 269.
- ³ Paracelsus: *De generatione stultorum*. Opp. Strasb., 1616, ii, 74.
- ⁴ Morel: *Du goitre et du cretinisme*. Etiologie, prophylaxie, traitement. Paris, 1864.
- ⁵ Koestl: *Der endemische Cretinismus als Gegenstand der öffentlichen Fürsorge*. 1855, Wien, 179 pp.
- ⁶ Gull, W.: On a cretinoid state supervening in adult life in women. *Trans. Chir. Soc.*, 1874, vii, 180.
- ⁷ Kocher, T.: Ueber Kropfextirpation und ihre Folgen. *Arch. f. klin. Chir.*, 1883, xxix, 254.
- ⁸ Murray, G. R.: Note on the treatment of myxedema by hypodermic injections of an extract of the thyroid gland of a sheep. *Brit. Med. J.*, 1891, ii, 796.
- ⁹ Seidell, A., and Fenger, F.: Seasonal variation in the iodine content of the thyroid gland. *J. Biol. Chem.*, 1913, xiii, 517.
- ¹⁰ Hirsch, A.: Endemic goiter and cretinism. *Handbook of geographical and historical pathology*. 2nd edit. New Sydenham Soc. Transl., 1885, ii, 121.
- ¹¹ Costa, S.: *Étude du goitre epidémique*. Thèse, Lyons, January, 1907.
- ¹² McCarrison, R.: An inquiry into the causation of goiter at Lawrence Military Asylum, Sanawar. *The Ind. J. Med. Res.*, 1914, i, 536.
- ¹³ Marine, D., and Lenhart, C. H.: Observations and experiments on the so-called thyroid carcinoma of brook trout (*salvelinus fontinalis*) and its relation to ordinary goiter. *J. Exp. Med.*, 1910, xii, 311.
- ¹⁴ McCarrison, R.: Observations on endemic goiter in the Chitral and Gilgit Valleys. *Med. Chir. Trans., Lond.*, 1906, lxxxix, 437.
- ¹⁵ St. Lager: *Etudes sur les causes du cretinisme et du goitre endémique*. Paris, Bailliere et fils, 1867.
- ¹⁶ McCarrison, R.: *The etiology of endemic goiter*. Bale, London, 1913.
- ¹⁷ McCarrison, R.: A review of the researches on endemic goiter. *Indian Med. Gaz.*, 1911, xlv, 253.
also *Collected papers on Goiter and Cretinism*, 1913-1914. Thacker, Spink & Co., Calcutta, 1915.
- ¹⁸ Chagas, C.: Nova entedu de morbida do homem. *Memorias do Instituto Oswaldo Cruz*, 1911, iii, 219.

- ¹⁹ Kollé, W., (a) quoted by McCarrison, R.: Etiology of endemic goiter. Bale, London, 1913.
(b) Reported by Langhans, T., and Wegelin, C.: Die Schilddrüse bei Infektion mit *Trypanosoma Cruzi*. Der Kropf der weissen Ratte. Haupt, Berne, 1919, p. 122.
- ²⁰ Dieterle, T., and Hirschfeld, L., and Klinger, R.: Epidemiologische Untersuchungen über den endemischen Kropf. Arch. f. Hyg., 1913, lxxx, 128.
- ²¹ Bircher, E.: Zur experimentellen Erzeugung der Struma, zugleich ein Beitrag zum deren Histogenese. Deutsch. Zeitschr. f. Chir., Leipzig, 1910, ciii, 276.
also Die Aetologie des endemischen Kropfes. Ergebn. der Chir. und Orthopaed., 1913, v. 133.
- ²² Wilms, M.: Experimentelle Erzeugung und Ursache des Kropfes. Deutsch. Med. Wehnschr., 1910, xxxvi, 604.
- ²³ Langhans, T., and Wegelin, C.: Der Kropf der weissen Ratte. Berne, 1919.
- ²⁴ Pliny: Guttur homini tantum et suibus intume scit aquarum, quae potantur plerumque vitio. Hist. Natural, lib., ix, Cap. 37, sect. 68.
- ²⁵ Barton, B. S.: A memoir concerning the disease of goiter as it prevails in different parts of North America. Phila., 1800.
- ²⁶ Marine, D., and Lenhart, C. H.: Further observations and experiments on the so-called thyroid carcinoma of brook trout (*salvelinus fontinalis*) and its relation to endemic goiter. J. Exp. Med., 1911, xiii, 455.
- ²⁷ Marine, D., and Lenhart, C. H.: On the occurrence of goiter (active thyroid hyperplasia) in fish. J. H. H. Bul., 1910, xxi, 95.
- ²⁸ Quoted by St. Lager, Loc. cit.
- ²⁹ Chatin, A.: Recherche de l'iode dans l'air, les eaux, le sol et les produits alimentaires des Alpes de la France. Gaz. des Hôpit., 1852, xxv, 14, 38, 50, 86, 94.
- ³⁰ Baumann, E.: Ueber das normale Vorkommen von Jod im Thierkörper. Zeitschr. f. Physiol. Chem., 1896, xxi, 319.
- ³¹ Baumann, E., and Roos, E.: Ueber das normale Vorkommen des Jods im Thierkörper. Zeitschr. f. Physiol. Chem., 1896, xxi, 481.
- ³² Baumann, E., and Goldmann, E.: Ist das Iodothyryn (thyroidin) des lebenswichtige Bestandtheil der Schilddrüse? Münch. Med. Wehnschr., 1896, xliii, 1153.
- ³³ Oswald, A.: Ueber den Jodgehalt der Schilddrüsen. Zeitschr. f. Physiol. Chem., 1897, xxiii, 265.
- ³⁴ Oswald, A.: Die Chemie und Physiologie des Kropfes. Virchow's Archiv., 1902, clxix, 444.
- ³⁵ Oswald, A.: Weiteres über das Thyreoglobulin. Beiträg. z. chem. Physiol. und Path., 1902, ii, 545.

- ³⁶ Kendall, E. C.: The isolation in crystalline form of the compound containing iodine which occurs in the thyroid; its chemical nature and physiological activity. Jour. Am. Med. Assoc., 1915, lxiv, 2042.
also The active constituent of the thyroid, its isolation, chemical nature and physiologic action.
Collected papers of the Mayo Clinic, 1916, viii, 513.
- ³⁷ Marine, D., and Williams, W. W.: The relation of iodine to the structure of the thyroid gland. Arch. Int. Med., 1908, i, 349.
- ³⁸ Marine, D., and Lenhart, C. H.: Further observations on the relation of iodine to the structure of the thyroid gland in the sheep, dog, hog, and ox. Arch. Int. Med., 1909, iii, 66.
- ³⁹ Marine, D., and Lenhart, C. H.: Relation of iodine to the structure of human thyroids. Relation of iodine and histological structure to diseases in general; to exophthalmic goiter; to cretinism and myxedema. Arch. Int. Med., 1909, iv, 440.
- ⁴⁰ Marine, D.: On the occurrence and physiological nature of glandular hyperplasia of the thyroid (dog and sheep) together with remarks on important clinical (human) problems. J. H. H. Bull., 1907, xviii, 359.
- ⁴¹ Marine, D.: On the physiological nature of the "Glandular Hyperplasias of dogs' thyroids with a detailed report of a case typical of the group. J. Infect. Dis., 1907, iv, 417.
- ⁴² Marine, D., and Lenhart, C. H.: Colloid glands (goiters); their etiology and physiological significance. J. H. H. Bul., 1909, xx, 131.
- ⁴³ Marine, D., and Lenhart, C. H.: Effects of the administration or the withholding of iodine containing compounds in normal, colloid or actively hyperplastic (parenchymatous) thyroid of dogs. Some experiments on (congenital) parental thyroid hyperplasia in dogs; remarks on the clinical manifestations associated with marked thyroid hyperplasia. Arch. Int. Med., 1909, iv, 253.
- ⁴⁴ Marine, D.: The rapidity of the involution of active thyroid hyperplasias of brook trout following the use of fresh sea-fish as a food. J. Exp. Med., 1914, xix, 376.
- ⁴⁵ Marine, D., and Johnson, A. A.: Experimental observations on the effects of the administration of iodine in three cases of thyroid carcinoma (two human and one canine). Arch. Int. Med., 1913, xi, 288.
- ⁴⁶ Marine, D., and Feiss, H. O.: The absorption of potassium iodide by perfused thyroid glands and some of the factors modifying it. J. Pharmacol. & Exp. Therap., 1915, vii, 557.
- ⁴⁷ Marine, D.: Quantitative studies on the *in vivo* absorption of iodine by dogs' thyroid glands. J. Biol. Chem., 1915, xxii, 547.
- ⁴⁸ Manley, O. T., and Marine, D.: Transplantation of ductless glands with reference to permanence and function. J. Am. Med. Assoc., 1916, lxvii, 260.

- ⁴⁰ Halsted, W. S.: An experimental study of the thyroid gland of dogs with especial consideration of hypertrophy of this gland. *J. H. Hosp. Repts.*, 1896, i, 373.
- ⁵⁰ Edmunds, W.: Experimental congenital thyroid hyperplasia in pups (in his Erasmus Wilson Lectures on the pathology of the thyroid) *Lancet*, Lond., 1901, i, 1451.
- ⁵¹ Carlson, A. J.: On the cause of congenital goiter (thyroid hyperplasia) in dogs and cats. *Am. J. Physiol.*, 1914, xxxiii, 143.
- ⁵² Hunt, R.: Experiments on the relation of the thyroid to diet. *J. Am. Med. Assoc.*, 1911, lvii, 1023.
- ⁵³ Watson, C.: The influence of a meat diet on the thyroid gland in the second generation of meat fed rats. *Proc. Physiol. Soc., J. Physiol.*, 1906, xxxiv, pp. xxix.
- ⁵⁴ McCarrison, R.: The effects of some food deficiencies and excesses on the thyroid gland. *Indian J. M. Res.*, 1920, vii, 633.
- ⁵⁵ Mellanby, E. and Mellanby, M.: The experimental production of thyroid hyperplasia in dogs. *Proc. Physiol. Soc., J. Physiol.*, 1921, lv, pp. vii and viii.
- ⁵⁶ Shapiro, S., and Marine, D.: Unpublished Data.
- ⁵⁷ Tait, L.: Enlargement of the thyroid in pregnancy. *Edinb. M. J.*, 1875, xx, 993.
- ⁵⁸ Murlin, J. R.: The metabolism of development. I. Energy metabolism in the pregnant dog. *Am. J. Physiol.*, 1910, xxvi, 134.
- ⁵⁹ Baer, J. L.: Basal metabolism in pregnancy and the puerperium. *Am. J. Obstet. & Gynec.*, 1921, ii, 249.
- ⁶⁰ Root, H. F., and Root, H. K.: The basal metabolism during pregnancy and the puerperium. *Arch. Int. Med.*, 1923, xxxii, 411.
- ⁶¹ Marine, D., Cipra, A., and Hunt, L.: Influence of the thyroid gland on the increased heat production occurring during pregnancy and lactation. *J. Med. Res.*, 1923, iv, in press. Also *Trans. Assoc. Am. Phys.* 1924, xxxix, 180.
- ⁶² Olin, R. M.: Iodin deficiency and prevalence of simple goiter in Michigan. *Jour. Am. Med. Assoc.*, 1924, lxxxii, 1328.
- ⁶³ McClendon, J. F., and Williams Agnes: Simple goiter as a result of iodine deficiency. *Jour. Am. Med. Assoc.*, 1923, lxxx, 600.
- ⁶⁴ Virchow, R.: *Die krankhaften Geschwülste*, Berl., 1863, iii, 4.
- ⁶⁵ Marine, D., and Lenhart, C. H.: The pathological anatomy of the human thyroid gland. *Arch. Int. Med.*, 1911, vii, 506.
- ⁶⁶ Marine, D., and Lenhart, C. H.: Pathological anatomy of exophthalmic goiter. The anatomical and physiological relations of the thyroid gland to the disease; the treatment. *Arch. Int. Med.*, 1911, viii, 265.
- ⁶⁷ Kocher, A.: Die histologische und chemische Veränderung der Schilddrüse bei Morbus Basedowii und ihre Beziehung zur Funktion der Drüse. *Virchow's Arch.*, 1912, ccviii, 86.

- ⁶⁵ Wegelin, C.: Zur Histogenese des endemischen Kropfes. *Correspondenz-Bl. f. Schweiz. Aerzte*, Basel, 1912, xlii, 321 and 376.
- ⁶⁹ Woelfler, A.: Ueber den embryonalen Aufbau der Schilddrüse. *Monograph*, Berl., 1880.
also Ueber die Entwicklung u. den Bau des Kropfes. *Arch. f. klin. Chir.*, 1883, xxix, 1 and 754.
- ⁷⁰ Marine, D.: Benign epithelial tumors of the thyroid gland. *J. Med. Res.*, 1913, xxvii, 229.
- ⁷¹ Marine, D.: Further observations and experiments on goiter (so-called thyroid carcinoma) in brook trout (*salvelinus fontinalis*) iii, Its cure and prevention. *J. Exp. Med.*, 1914, xix, 70.
- ⁷² Smith, G. E.: Fetal athyreosis. A study of the iodine requirements of the pregnant sow. *J. Biol. Chem.*, 1917, xxix, 215.
- ⁷³ Hart, E. B., and Steenbock, H.: Thyroid hyperplasia and the relation of iodine to the hairless pig malady. *J. Biol. Chem.*, 1918, xxxiii, 313.
- ⁷⁴ (a) Unpublished data in collaboration with Mr. John Ronayne of Pemberton Meadows, B. C. during 1916-1917.
(b) Keith, W. D.: Endemic goiter in British Columbia. *Canad. Med. Assoc. J.*, 1924, xiv, 284.
- ⁷⁵ Marine, D., and Kimball, O. P.: The prevention of simple goiter in man. A survey of the incidence and types of thyroid enlargements in the school girls of Akron (Ohio) from the 5th to the 12th grades inclusive. The plan of prevention proposed. *J. Lab. & Clin. Med.*, 1917, iii, 40.
- ⁷⁶ Marine, D., and Kimball, O. P.: Prevention of simple goiter in man. *Arch. Int. Med.*, 1920, xxv, 661.
- ⁷⁷ Klinger, R.: Die Prophylaxe des endemischen Kropf. *Schweiz. Med. Wehnschr.*, 1921, li, 12.
- ⁷⁸ Fritzsche, E.: Radikale Kropfoperation und Kropfprophylaxe. *Schweiz. Med. Wehnschr.*, Basel, 1921, li, 1016.
- ⁷⁹ Bayard, O.: Ueber das Kropfproblem. *Schweiz. Med. Wehnschr.*, Basel, 1923, liii, 703, 732.
- ⁸⁰ Hunziker, H., and von Wyss, M.: Ueber systematische Kropf Therapie und Prophylaxe. *Schweiz. Med. Wehnschr.*, 1922, lii, 49.
- ⁸¹ de Quervain, F.: La prophylaxie du goitre. *Rev. d'hygiene*, 1922, iie annee, 227.
- ⁸² Muggia, G.: L'opera del Comitato Provinciale Valtellinese per la lotta contra il gozzo nell'anno scolastico, 1922-23. Sondrio, 1923.
- ⁸³ Pighini, G.: Per la profilassi della endemia gozzo-cretinica. *Difesa Sociale*, 1923, ii, 4.
- ⁸⁴ Commission suisse du goitre. Supplement au "Bulletin du Service federal del'hygiene publique." 1923, No. 5, p. 19.

- ⁸⁵ von Humboldt, A.: Observations sur quelques phenomenes peu connu qui offre le goitre sous les tropiques dans les plaines et sur les plateaux des Andes. Jour. de Physiologie experimentale et Pathologique, 1824, iv, 109.
- ⁸⁶ Roulin: Sur quelques faites relatifs a l'histoire des goitres. Jour. de Physiol. experim. et Path., 1825, v, 266.
- ⁸⁷ Crawford, Mrs. Lucy: History of the White Mountains (New Hampshire). 2nd edit., Hoyt, Fogg & Co., Portland, Me., 1883.
- ⁸⁸ Marine, D.: The importance of our knowledge of thyroid physiology in the control of thyroid diseases. Arch. Int. Med., 1923. xxxii, 811.
- ⁸⁹ Goldberg, S. A., and Simpson, S.: Certain pathological tissue changes in thyroidectomized sheep. Proc. Soc. Exp. Biol. & Med., 1924, xxi, 567.

ALTERATIONS OF INTRAPLEURAL PRESSURE AND THEIR SIGNIFICANCE¹

DR. EVARTS A. GRAHAM

Professor of Surgery, Washington University School of Medicine,
St. Louis

THE danger of an open pneumothorax was apparently common knowledge to the ancient Greeks. Celsus,² in discussing the question of studying anatomy by dissection of living criminals, a practice in vogue in Alexandria in the third century before Christ, states: "It is indeed true that the abdomen, with which our argument is less concerned, can be opened while a man yet lives, but as soon as the knife reaches the thorax, and cuts the transverse septum, which is a membrane dividing the superior parts from the inferior and called diaphragma by the Greeks, the man at once gives up the ghost, and thus it is the breast and its viscera of a dead and not a living man which the murderous physician examines. He has thus but performed a cruel murder, and has not learned what the viscera of a living man are like." Despite the antiquity of the knowledge that incisions cannot be made in the thorax with the same impunity as in the abdomen, our present knowledge of the processes involved are by no means complete, and in recent years even the idea that a thoracic opening is not so innocuous as an abdominal one has been repeatedly challenged. The problem, although a physiological one, has been given but scant attention by the physiologists. To the clinician it is a matter of prime importance to know in an exact manner what effects may arise from alterations in the intrapleural pressure; for some of the most common conditions are associated with some of these effects. To the thoracic surgeon it is essential that he be familiar at least with the grosser effects of a pneumothorax if he wishes to minimize his disasters.

¹ Delivered February 9, 1924.

Under normal conditions each lung completely fills its corresponding pleural cavity except for the presence of a very small amount of fluid; there is, therefore, only a potential pleural space. But at the same time that each lung completely fills its pleural space, its elastic tissue is tending to contract and to pull the lung away from the chest wall. It is therefore obvious that if the pressure between the lung and the chest wall be measured under ordinary conditions it will be found to be "negative," that is, it will be less than that of the atmosphere. The essential mechanical factor in inspiration is an enlargement of the thorax. This has a tendency to pull the chest wall away from the lung with the result that the intrapleural pressure becomes more "negative," that is, still less than that of the atmosphere. It is obvious therefore that the intrapleural pressure just at the end of a deep inspiration is lower, that is more "negative," than at any other time. Conversely, at the end of an expiration it is higher than at any other time, that it is less "negative." Under ordinary normal conditions the intrapleural pressure does not become equal to or greater than the atmospheric pressure. Negative pressure at some time during the act of inspiration is necessary in order that air should rush down the trachea to fill the lungs.

Donders³ was apparently the first to measure the intrapleural pressures in the human. At the end of quiet expiration he found a pressure of -7.5 mm. Hg. and at the end of inspiration -9 mm. Hg. Aron,⁴ in a living normal man, found at the height of quiet inspiration an average reading of -4.64 mm. Hg. and at the height of quiet expiration -3.02 mm. Hg. It is natural to expect that a normal variation in the intrapleural pressure will occur. There is therefore no absolute normal pressure reading for either inspiration or expiration. The literature on this subject is extensively reviewed in the exhaustive monograph of Emerson.⁵

Abnormal conditions which may disturb the intrapleural pressures result from the presence of fluid, gas or tumors in the pleural space. Although all of these will exercise pressure on



FIG. 1.—Picture of the normal thorax of an adult man who died of peritonitis taken one hour after death. The trachea shows plainly in the mid-line.



FIG. 2.—The same man after air had been introduced into the left pleural cavity until a pressure of only 8.5 cm. of water (only 0.65 cm. of mercury) was obtained. Even with this slight alteration of pressure there is a marked deflection of the trachea to the right, and the position of the heart is correspondingly changed. This picture clearly illustrates the ease with which relatively marked displacements of the thoracic viscera are produced by slight alterations of intrapleural pressure.

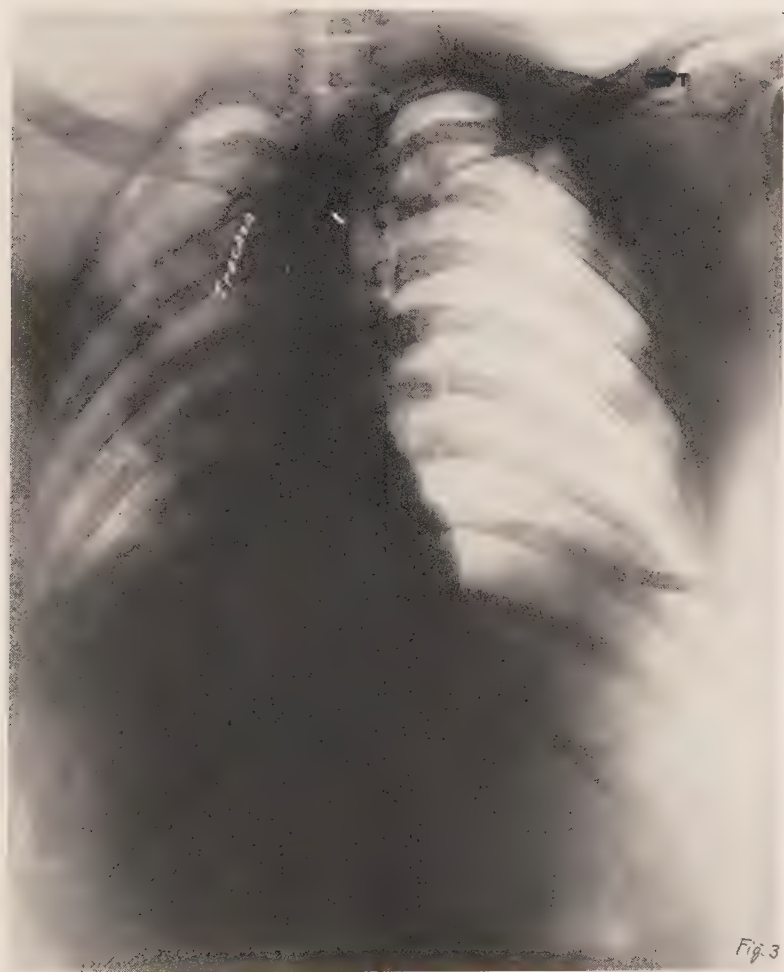


FIG. 3.—The same man as the preceding after enough air was injected into the left pleural cavity to make a pressure equal to 33 cm. of water (2.54 cm. of mercury). Only a small portion of the heart remains in the left pleural cavity.



FIG. 4.—The same man as the preceding. Air has been introduced into the left pleural cavity until a pressure equal to that of 54 cm. of water (4.15 cm. of mercury) was obtained. Both lungs now occupy the right half of the thorax, and the heart has become rotated. Free air is visible at the right of the mid-line so that there is an apparent bilateral pneumothorax.

the lung, each operates in a slightly different manner. In the case of fluid the greatest pressure will be exerted at the bottom of the pleural space, in accordance with the laws of hydrostatic pressure. Gas, on the other hand, will exert an equal pressure in all parts of the pleural cavity, provided that there are no adhesions to obliterate any of the space; and a tumor will exert its chief pressure wherever it happens to be located. It is therefore apparent that conclusions concerning the mechanics of the thorax which are based on observations made on the effects of fluid in the chest are not necessarily applicable to a consideration of the effects of air in the pleural space. The failure to recognize this fact has been responsible for much of the confusion in the literature on this subject.

To the surgeon the greatest interest is concerned with the effects of air in the pleural cavity because anyone who performs intrathoracic operations should be familiar with the effects which result from an opening in the chest wall. To the physiologist likewise, a study of the effects of a pneumothorax reveals important facts concerning both respiration and the circulation. Limitations of time will compel me to discuss chiefly in this paper the effects of a pneumothorax.

Until very recent times an accidental admission of air into the pleural cavity through a free opening was generally regarded with great dread because of the possibility of immediate death. Probably no other cause contributed to such a degree to retard the development of thoracic surgery as the fear of the consequences of making a free opening into a pleural cavity. To most writers on this subject any opening into a pleural cavity which permitted the free entrance of air signified an immediate collapse and loss of function of the lung on that side; the size of the opening was considered to be a matter of little or no consequence. Curiously confused with this idea was also the prevalent conception that, even in a normal chest, the mediastinal structures constitute a more or less rigid partition between the two pleural cavities. The literature on this subject is extensively reviewed in the articles by Emerson,⁶ Sauerbruch⁷ and L. Mayer.⁸ Although

the flapping of the mediastinal structures to and fro in a condition of open pneumothorax was frequently mentioned, there was but little realization of the extent to which the lung of the unopened pleura was handicapped.

A matter of great controversy at the present time, particularly among thoracic surgeons, is the question of just how much the opposite lung is affected when an opening is made in one pleural cavity. Much of this controversy unfortunately is concerned with opinions and conjectures rather than with actual pertinent data. Rational experimental investigation of pneumothorax apparently began with Hewson⁹ in 1767 who tried to produce it in animals by injury of the lung through the chest wall. Cruveilhier¹⁰ found in 1836 that both pleural cavities of the dog may be opened simultaneously without a fatal result. This contradicted the earlier conception of Van Swieten that a bilateral open pneumothorax is necessarily fatal if the area of the combined openings is larger than that of the glottis. Since then until the last decade experimental studies of the effects of pneumothorax have been relatively few and sporadic.

In 1918 R. D. Bell and the writer,¹¹ while members of the U. S. Army Empyema Commission, undertook an experimental investigation of the mechanics of pneumothorax, particularly with reference to the question of the effects of open drainage in cases of empyema. One of the methods of experimentation was the injection of air into one pleural cavity until a pressure of 10 cm. of water was obtained and observation of the resulting change of intrapleural pressure on the other side. The experiments were first conducted on human cadavers immediately after death and on normal dogs killed with ether. No marked difference between the dead dog and the dead human was observed, when the thorax was normal in the sense that there were no adhesions and no thickening of the mediastinal structures. Accordingly experiments carried out on the living dog seemed applicable to the living human. In the course of these experiments it was found that if an air pressure in one pleural cavity equal to that of 10 cm. of water is obtained, the pressure in the other cavity will



FIG. 5.—Left pneumothorax in a newly born baby due probably to an accidental needle puncture of the lung from an injection of adrenalin into the heart. Soon after birth the baby's heart stopped beating but the intracardiac injection of adrenalin revived it for a few hours. At the time this picture was taken the baby was alive, despite the fact that apparently both lungs and the heart are crowded into the right half of the thorax. The aspiration of the air from the left pneumothorax greatly improved the respiration.

be nearly the same, differing by only two or three centimeters. It was concluded, therefore, that from the standpoint of pressure relationships, the thorax can be considered practically as one cavity and that any alteration of pressure in one pleural cavity will show a change in the opposite one of practically the same degree. The sudden interruption of the work because of transfer to an overseas unit prevented further work with the use of higher pressures. It is only recently that a resumption of the work has shown that when higher pressures are used for experimentation there is a greater and greater difference between the pressures of the two sides in the case of the human and that therefore we were wrong in our former conclusion that any alteration of pressure in one pleural cavity will show a change in the opposite one of practically the same degree, although the previous observations dealing with low pressures were correct. The details of these more recent experimental results will be discussed later (page 147).

Other conclusions which we drew from our earlier work were as follows:

(1) Older conceptions are erroneous which imply that in pneumothorax only one lung is affected and that that lung is rendered functionless, regardless of the size of the opening.

(2) On the contrary both lungs are affected; and, when a small pneumothorax is present, they are both affected to nearly the same extent. Moreover, a lung does not necessarily become functionless merely because there is a free communication between the pleural cavity of that side and the outside air. If such were the case then a bilateral open pneumothorax would promptly result in death from asphyxia. As a matter of fact, however, a bilateral open pneumothorax in an experimental animal is well tolerated and is compatible with life for an indefinite period, provided that the bilateral openings are not too large. I have found this fact to be true in both the dog and the rabbit.

(3) A unilateral open pneumothorax will result in death if the opening be made sufficiently large.

(4) The really important matter is the amount of air which

enters through the opening at each inspiration and the ability of the individual to compensate for the encroachment on his respiratory surface which is caused by the pneumothorax. Roughly speaking this relationship becomes a question of the relation of the size of the opening and the value of the vital capacity of the individual. When the vital capacity is high a larger opening can be tolerated than when it is low; and no opening at all of any appreciable size can be withstood if the vital capacity is so low that it practically equals the tidal air. In other words, even a very small opening will result in a fatal asphyxia, if, before the opening is made, the vital capacity is so low that in spite of maximal inspiratory efforts only enough air is inhaled to satisfy the tidal air requirements. A mathematical expression was given to show how a calculation could be made of a maximum non-fatal opening in a given case if the vital capacity was known. This expression was based on the assumption that the pressures in the two pleural cavities in the normal chest were always nearly equal even in the presence of a unilateral open pneumothorax.¹² It was stated, however, that the value could be only an approximation because of the variability of some of the factors involved.

(5) Obviously, the conclusions mentioned above are concerned only with relationships as they exist in a normal thorax, that is one without adhesions or inflammatory induration. If sufficient adhesions exist or if the mediastinal pleura has been made rigid by inflammatory induration, as for example in cases of chronic empyema, then the relationships discussed above no longer hold true, and no ill effects from pressure disturbances will result even if a very large opening is made.

Both the experimental observations briefly summarized above and the conclusions drawn from them have been questioned by several writers. There is, for example, a frequently quoted statement in the literature that the dog differs radically from the human in that normally there is a communication between the two pleural cavities of the dog, while in the human there is no such communication. This statement has been made by Matas,¹³ Duval,¹⁴ Yates¹⁵ and others. More recently Snyder¹⁶

has claimed that it is impossible to produce a unilateral pneumothorax in the dog because of the alleged communication between the two pleural cavities. I have examined twenty-five dogs with the particular purpose of finding out whether there are any anatomical openings in the mediastinal pleura and have never found any. It is interesting also that very recently Duval¹⁷ and Matas¹⁸ have retracted their former opinions about the presence of a normal communication between the two pleural cavities of the dog. Snyder¹⁶ has stated that in the dog it is impossible to create a unilateral pneumothorax because the air passes at once from one pleural cavity through the mediastinum to the other one with the result that the pneumothorax is bilateral. He shows also that fluids pass readily from one side to the other, and he quotes Garland¹⁹ and Calvert²⁰ as having previously called attention to this fact. Both Duval¹⁷ and Matas,¹⁸ although now admitting the absence of any anatomical communication between the two pleural cavities in the dog, nevertheless maintain that in that animal the mediastinal septum is so thin and tenuous in comparison with that of the human that experimental observations of pleural pressures made in the dog are not applicable to the human. In our original article in 1918 we had concluded that they were applicable in all particulars. In reply to this phase of the controversy I wish to state that I have repeatedly produced a unilateral pneumothorax in the dog with marked deflection of the heart and mediastinal structures into the opposite pleural cavity and I have published the confirmatory X-ray reproductions.²¹ A reopening of the study, however, has convinced me that in many dogs the pneumothorax does soon become bilateral and that there is a considerable difference between even apparently normal dogs in the amount of resistance to pressure offered by the mediastinum. In some dogs simultaneous readings of the pressures in the two pleural cavities after the injection of air into one give results very similar to those in the human; but in others the results are such as would be expected in a bilateral pneumothorax. Even if one concludes that the dog may not be the animal entirely suited for

the experimental study of *unilateral* pneumothorax, it would seem nevertheless that this animal would be suitable for the study of the phenomena associated with *bilateral* pneumothorax and that the result obtained in such a study would be applicable to a consideration of bilateral pneumothorax in the human. Even if one admits that the mediastinal septum in the dog is more easily stretched, more easily torn and more easily penetrated by gases and fluids than is the human, it is nevertheless perhaps not out of place to call attention to certain irregularities in the methods of experimentation. If one wishes to measure the pressure in the opposite pleural cavity after injecting air into one, it is necessary to permit the entrance of a little air into the opposite cavity in order to have a cushion of air around the canula which will permit a reading. If this is not done, the lung will usually force itself against the end of the canula and prevent any oscillation of air within the canula and tube. One starts therefore with some degree of bilateral pneumothorax which probably results in the retraction of the lung away from the mediastinal pleura. The final result is that if the lung is retracted away sufficiently the mediastinal septum will be hung in space at its edges without the buttressing support of the lung against it. It is obvious then that a thin mediastinal partition will be ruptured more easily under such circumstances than under the conditions which are presented in the case, for example, of a surgical unilateral pneumothorax. For in the latter the only air which can be present in the opposite pleural cavity will be that which may have diffused through the mediastinum; moreover the mediastinum will be everywhere supported by being buttressed against the opposite lung. What we are really desirous of knowing, therefore, is not merely what the resistance of the mediastinal partition is, but also how much is the other lung compressed by force transmitted through the mediastinum. Nearly all of the experimental observations that have been published are really concerned only with the question of the resistance of the mediastinum.

Even the admission on my part that experimental observa-

tions on unilateral pneumothorax in the dog may not be applicable to the human in their entirety does not in any way annul the chief conclusions to be drawn from our earlier work, although these have been severely criticized by Duval.¹⁴ He states, for example, that the size of the pleural opening is not a matter of great importance as regards the possibility of causing death. He is a strong advocate of a very large incision for the expressed purpose of making a total unilateral collapse of the lung to permit no respiration at all on that side and no "ventilation" of the pleura. He desires to make that lung completely airless. He states that this procedure also prevents the circulation of "pendelluft," a term used by Brauer to indicate the theoretical transfer of air from the unaffected lung into the partly compressed lung at expiration and the rebreathing of this air into the uncompressed lung on inspiration. Brauer's idea was of course that this phenomenon was an important factor in the production of asphyxia because this air would have its oxygen diminished besides containing an excess of CO₂ and would also prevent the inspiration of that much fresh air. Duval claims that the thorax of the bovine class of animals resembles that of the human and that an animal of this class should be used for experiments instead of the dog. He states that an experiment which he performed on a calf lends support to his ideas. He considers that in order to make a very large opening safe, all that is necessary to do is to immobilize the corresponding side of the chest and diaphragm by means of a powerful automatic retractor and to express from the lungs its residual air. To accomplish this he delivers the lung out of the wound. He also recommends enveloping and progressively compressing the lung with warm gauze compresses. He regards the "ventilation" of the pleura as the most serious and harmful factor resulting from an open pneumothorax. By this he means presumably the passage of air in and out of the pleural cavity. In his criticism of our work he makes no reference at all to the importance of the vital capacity of the patient, a point which we strongly

emphasized. I²² have already replied to Duval's criticisms in a separate article; but I shall discuss some of his points and conclusions here.

It is rather generally admitted that a unilateral open pneumothorax may sometimes have disastrous results. Duval's conclusions differ diametrically from mine as to the reason for these disasters. He considers them as being due to the fact that the opening is not large enough, whereas on the contrary, the evidence seems to me to point to the fact that they arise because in a given case the opening has been too large. From the practical standpoint of surgical methods it is of the utmost importance to settle this question definitely. For that reason it will be well to analyze with some care the effects of a pneumothorax in order to gain a clearer insight into this important question.

The majority, if not all, of investigators who have used animals for the experimental study of pneumothorax have observed a change in the character of the respiration after the creation of an open pneumothorax. Likewise most surgeons of wide experience in thoracic work whose observations have been carefully made have noticed a change in the character of the respiration in the human in the presence of an open pneumothorax. Usually the first alteration is an increase in the depth of inspiration (an increase in amplitude), followed later by an increase in the rate. In an earlier paragraph I have already pointed out that the act of getting air into the lungs consists chiefly of enlarging the thorax, a procedure which necessarily reduces the intrapleural pressure (makes it more negative) and thus causes air to rush down the trachea to inflate the lungs. We should expect similarly that whenever a sudden interference with the intrapleural pressure occurs which would make it equal to that of the atmosphere, the natural compensatory mechanism to offset this would be an attempt to create again a negative intrapleural pressure in order to get air into the lungs. The simplest way to accomplish this is to enlarge the thorax still more in an effort to reduce its interior pressure. This is just what happens then as

an automatic effort to counteract the sudden increase in the pleural pressure. This reveals itself in an increase in the depth or amplitude of the respiration. For purposes of simplicity we can compare in this respect the lungs and pleural cavities with any object containing a gas, as for example, a toy balloon. If the balloon should suddenly be doubled in size, or if the gas were suddenly transferred to a balloon of twice the size of the original one, the pressure of the contained gas would be only one-half of what it had been originally. In a structure like the thorax which is constantly changing its size, an opening communicating with the outside air does not necessarily imply that the pressure within is always equal to that of the atmosphere, especially if the opening is a small one. This has been a common source of confusion. During inspiration the intrapleural pressure is usually negative and during expiration it is positive, if the opening is not too large.

Haldane and Poulton ²³ have shown that if an individual is placed in an atmosphere containing 4 to 5 per cent. of CO_2 his respiration increases in depth and little by little increases in rapidity. On the other hand, if the amount of inspired oxygen is suddenly reduced below 14 per cent. there is also at first an increase of amplitude of respiratory movements which they think is to be attributed to the fact that the anoxemia, by lowering the threshold of the respiratory center for CO_2 provokes the expulsion of CO_2 from the body. At the same time the rate is accelerated as the result of the anoxemia. Finally the respiration becomes periodic. If one continues to reduce the percentage of oxygen in the inspired air to 10 per cent., the respiration becomes superficial and finally death occurs. Haldane, Meakins and Priestley ²⁴ more recently have shown that if, instead of lowering the partial pressure of oxygen in the inspired air, one leaves the air at its normal composition but diminishes the amount of air entering the lungs sufficiently to produce a superficial respiration, the respiration soon becomes periodic. Periodic respiration is known always to be due to lack of oxygen. The anoxemia

therefore induces superficial respiration which itself maintains the state of anoxemia. A serious vicious circle is therefore established.

Now Duval states that in a unilateral open pneumothorax dyspnea exists as long as the corresponding lung respire partially and "dances" a little in the thorax, that is, as long as the external air strongly ventilates the pleura, but that as soon as that lung is put into a condition of complete collapse the dyspnea disappears. I have not been able to confirm this observation although, in accordance with Duval's recommendation, I used a calf for experiment. Moreover Dautrebande and Spehl²⁵ fail to agree with Duval in spite of the fact that they used rabbits, animals which like the bovine group, Duval recommends for experimental use because of their similarity to the human. In fact, in direct contradiction to Duval's claims I found that a calf dies if a large unilateral opening is made in the chest. It is difficult to understand how there could be any disagreement on a point so easy to demonstrate experimentally. When a large opening is made, however, the respiration soon becomes much more superficial, a fact which perhaps Duval has interpreted as a reduction in the dyspnea. But this is not to be regarded as a favorable sign. On the contrary, as Dautrebande and Spehl have shown in their excellent article, it should be looked upon as a danger signal because it indicates an anoxemia. This conclusion would agree very well with the work of Haldane and his pupils to which reference is made above. Accordingly Dautrebande and Spehl state that they regard Duval's conclusions as erroneous.

As has been mentioned above, Duval regards a large opening in the chest as less dangerous than a small one. My own opinion, however, based on many experimental and clinical observations is that, other things being equal, the larger the opening the greater the danger to life. The reasons for this conclusion are many. In the first place, it has been a frequent observation that animals will endure a bilateral open pneumothorax almost indefi-

nately if the openings in each side of the chest are not too large. In the case of dogs and cats of ordinary size, for example, if a small trocar is inserted into each pleural cavity the animal will go on for hours with often no evidence of any disturbance except for an increase in the amplitude of the respiratory movements, and this disturbance will promptly disappear after the withdrawal of the trocars. If, however, two large openings are made the animal will soon die with the picture of asphyxia. It seems to me that this simple experiment permits several important conclusions. It shows first of all that a lung does not become functionless merely because outside air is allowed free entrance to the pleural cavity. It shows also very clearly that the important consideration is the size of the openings. Other subsidiary factors in the production of death, to which great importance has been attached by some, such as "pendelluft" and the fluttering of the mediastinum, are ruled out in a case of bilateral open pneumothorax induced by openings of the same size which has proved fatal; for in such a case since the pressures are equal in both pleural cavities at all times there will be no to and fro motion of the mediastinum and also no chance for the occurrence of "pendelluft," and yet the animal dies. Now when we come to consider the question of a unilateral open pneumothorax we find that practically the same phenomena occur. The principles involved are substantially the same as are involved in a bilateral opening because the effects of a unilateral opening are not confined to only one lung but concern both lungs. The details, however, are slightly different, because, as has been said above, the pressures in the two pleural cavities during a unilateral open pneumothorax are not quite the same and because there is some to and fro movement of the mediastinum, etc. Nevertheless, in view of what we have just said about a bilateral open pneumothorax, it seems reasonable to suppose that with a unilateral opening such questions as the fluttering of the mediastinum and the action of "pendelluft" are of less importance than the direct effects on the lungs caused by the altered pres-

tures. This brings us back again to the importance of the size of the openings.*

The usual attempt to compensate for the increased pleural pressure is to take a deeper inspiration, as has been discussed above. Theoretically all that is necessary to do to prevent a fatal asphyxia is to get enough air into the lungs to satisfy the tidal air requirements. This can be accomplished of course in spite of an open pneumothorax provided that too much air does not enter the pleural cavity with each inspiration. If so much air enters the pleural cavity through the open pneumothorax wound that pulmonary inflation is sufficiently compromised to prevent the possibility of inhaling enough air into the lungs to maintain life, then of course a fatal asphyxia will occur. Fortunately here, as in other respects, nature has made provision for a generous reserve. The tidal air requirements ordinarily constitute only about one-eighth to one-twelfth of the total amount of air which can be inhaled if a maximal effort is made. This latter

* In a recent article Lundsgaard and Van Slyke²⁶ invoke an idea similar to that of Brauer's "pendelluft" as an explanation of cyanosis in unilateral open pneumothorax. They quote experiments by Sackur²⁷ who after producing a supposed collapse of one lung in rabbits and in a dog by unilateral open pneumothorax found a diminished content of oxygen in the arterial blood. On the basis of these experiments they conclude that cyanosis in acute unilateral open pneumothorax is produced "in the same manner as when the air passages to one lung are blocked, viz., by blood flow through unaerated lung space." In considering the pressure effects of open pneumothorax as if only one lung were concerned, they show that they are unaware of the fact that these effects involve both lungs. This leads them to the assumption that the frequent absence of cyanosis in spontaneous pneumothorax in tuberculosis is due perhaps to extensive thrombosis of the vessels in the lung on the involved side, a condition which they infer would prevent cyanosis because a relatively small amount of blood could pass through that lung. It seems to me just as logical to assume that the explanation of the absence of cyanosis in such a case lies in the fact that because of the presence of adhesions and fixation of the mediastinum there are no serious pressure effects on the opposite lung and that therefore actually a less amount of lung tissue is involved than if no adhesions were present, to say nothing of the altered relationships of the heart and large vessels when a pneumothorax is induced in the absence of adhesions.

amount of air represents what is known as the vital capacity. It is apparent therefore that a very considerable amount of lung tissue can be made functionless and yet permit the individual to take in enough air to maintain life by increasing his respiratory movements. This then would seem to be the explanation of the well-established fact that life is compatible, in a vigorous adult, with a relatively large unilateral opening and even with bilateral openings whose combined areas greatly exceed the area of the opened glottis. A common error in the older literature on pneumothorax was that a lung becomes collapsed and functionless if a pleural opening as great or greater than the diameter of the glottis is made. A person who cannot make so great a respiratory effort cannot resist so large a pleural opening as the one who can make a great inspiration; likewise it becomes apparent that the same individual, if he has any serious impediment to his respiration, will die from a pleural opening which would not be fatal if the impediment were absent. In other words, therefore, the vital capacity of the individual is of great importance in determining the size of a pleural opening which is compatible with life. One who has a large vital capacity can withstand a larger pleural opening than one whose vital capacity is low. By considering what conditions lower the vital capacity we can understand readily that a large pleural opening is likely to be fatal in an individual whose respiratory muscles are weak, who has an uncompensated heart, who has an extensive pneumonia, a pulmonary edema, an acidosis, a deep general anesthesia, etc. Indeed, if he is so dyspneic that he just succeeds in maintaining life in spite of maximal respiratory effort, in other words if his vital capacity practically equals his tidal air requirements, he may succumb to the effects of even a very small pleural opening.

In some previous publications I²⁸ and ²⁹ have called attention to the fact that on purely theoretical grounds, even if an alteration of pressure in one pleural cavity induced a change in the other of the same degree it can be calculated that a pleural opening can be withstood of such a size that it would have seemed impossible before the war experience. For example, on the basis

of our calculation, a man with a high vital capacity (7180 c.c.) could support life with a unilateral opening as large as 15.6 square inches, or 101 square centimeters. These calculations, however, are not strictly accurate but are only approximations because of the variability of several of the factors. As a matter of fact, since the pressures in the two pleural cavities are not equal when there is great increase in one, it is seen that in some individuals with high vital capacities a unilateral opening considerably larger than 101 square centimeters may be compatible with life. It really is of little importance to know exactly in any given case how large an opening can be made. The important thing to realize is that those whose vital capacities are low, especially those who are already dyspneic, may die as a result of even a small opening unless measures are taken to prevent serious consequences.

The most important aid in minimizing the harmful effects of an open pneumothorax is to close the opening. Closure of the opening leads to an almost instantaneous improvement in the respiration. The explanation of the relative innocuousness of a closed as compared with an open pneumothorax is apparently as follows: When an opening is closed, particularly if it is closed at the end of expiration, there is not enough air remaining in the pleural cavity to encroach on the breathing surface of the lungs sufficiently to prevent the establishment of a negative intrapleural pressure by a slight increase in the depth of inspiration. There is no longer an active competition between the passage of air down the trachea on the one hand and the entrance of air into the pleural cavity on the other hand. The impossibility of getting the requisite amount of tidal air into the lungs does therefore not occur. Experimentally, however, a closed pneumothorax can be made to cause a fatal asphyxia if enough air is injected into the pleural cavity. Here again the process seems to consist fundamentally of increasing the intrapleural pressure to such an extent that it becomes impossible to create a negative pressure in spite of maximum respiratory efforts; the necessary amount of tidal air cannot be obtained, with

the inevitable result that asphyxia occurs. Again, moreover, if the vital capacity is low, it is easier to produce death with a closed pneumothorax for reasons which have been made sufficiently clear in the discussion given above. Doubtless some of the catastrophes which have occurred in the production of artificial pneumothorax for therapeutic purposes are explainable on the fact that patients with low vital capacities and without adhesions have been given too much air. An amount of air which might be well tolerated by a patient with a high vital capacity might be fatal in one whose vital capacity is low. The prompt withdrawal of air in an alarming case will usually improve the dyspnea and prevent death. It is an interesting fact that Duval and others 'who have criticized our theory of the action of pneumothorax recommend strongly the use of measures which tend to reduce the size of the opening when performing operations on the chest. They therefore, unconsciously support our theory while at the same time they attack it. For example, they frequently deliver the lung out of the incision; they also pack in gauze compresses. They lose sight of the fact that the size of the incision and the size of the opening are not synonymous when anything is in the incision, no matter whether it is a delivered lung, a gauze compress, the surgeon's hand, instruments or what not. A hole that is either partly or completely plugged obviously cannot be as large as if it contained nothing. Other aids in combating the asphyxial effects of an open pneumothorax are the administration of either oxygen or carbon dioxide, as recommended by Dautrebande and Spehl, to break up the vicious circle which occurs when the anoxemia appears. Oxygen will supply directly the thing that is most needed, but the administration of carbon dioxide will accomplish the same purpose indirectly by stimulating deeper inspirations and thereby the taking in of more air.

Since the explanation of the action of pneumothorax which I have summarized here has not met with universal acceptance, it will be worth while to see how well it harmonizes with the clinical facts which were brought out particularly during the war. One fact which perhaps stood out above all others in rela-

tion to war wounds of the chest was the unanimous agreement that it was important to close sucking (open) wounds promptly. The high mortality attending open wounds was attributed by some to the increased risk of infection. This was undeniably an important factor, but yet, according to Duval,³⁰ 50 per cent. of all fatal lung wounds died within the first day. It seems improbable that a severe enough infection could have manifested itself so soon as to be responsible for so high a mortality and likewise it is doubtful if hemorrhage could have caused most of the deaths. Is it not more probable that many, if not most, of these deaths occurred as a result of the pressure disturbances incidental to an open pneumothorax? This point is discussed at more length in our original article published in 1918. Again the fact that living men were often seen with wounds of the chest wall which were so large that they would have seemed before the war to be incompatible with life does not seem strange in the light of the theoretical discussion presented above. Finally also, in the case of acute empyema, it would seem to me that nothing more clearly substantiates the truth of the principles here set forth than the remarkable reduction in the mortality which followed the abandonment of the practice of early open drainage in the acute pneumonic stage of the disease. The explanation of the remarkable decrease in mortality accomplished by the postponement of open drainage seems easy in the light of the discussion given above. During the stage when pneumonia is present the vital capacity is low.¹¹ In some cases it was actually so low that in spite of maximal respiratory efforts it was difficult for the patient to take in enough air to maintain life. Moreover, at this time there was usually no walling off of pus in the pleural cavity. An open drainage, therefore, at this stage of the disease carried with it all the dangers of an open pneumothorax at a time when, in many cases, the vital capacity was so low that an opening of any size would have been fatal. Later, however, after the pneumonia had cleared up, not only had the vital capacity increased but also usually the pus had become more or less circumscribed by adhesions so that a drainage opening did not create a free pneumothorax. These remarks hold true

especially for the type of pneumonia and empyema due to the hemolytic streptococcus. I believe the principle of avoiding an open drainage during the stage of acute pneumonia is now universally accepted in this country. At Camp Lee we saw the mortality drop from more than 40 per cent. to less than 5 per cent. by carrying out this principle. Stone,³¹ at Fort Riley, showed a correspondingly marked reduction in the mortality after the early open drainage during the pneumonic stage was abandoned. The tabulation of his results is as follows:

1. First Series: Early operation (Oct. 20, 1917, to Jan. 21, 1918), eighty-five cases; mortality, 61.2 per cent.
2. Second Series: Early aspirations and late operation (Jan. 12, to Aug. 10, 1918), ninety-six cases; mortality, 15.6 per cent.
3. Third Series: Early aspirations and later operation (Oct. 18, 1918, to Feb. 14, 1919), ninety-four cases; mortality, 9.5 per cent.

At the St. Louis Children's Hospital, since September, 1919, we have treated 90 cases of acute empyema by a plan of repeated aspirations during the pneumonic stage to be followed by free drainage when necessary. In this series there have been eleven deaths, but in ten of these there were serious complications, such as suppurative mastoiditis or meningitis, etc. This mortality of 12 per cent. compares very favorably with that of 54 per cent. given by Holt³² in a series of 126 cases in children reported before the war. The empyema records of the St. Louis Children's Hospital before the war are not available.

There are also now a considerable number of investigators who have carried out experiments since the publication of our paper in 1918 whose results in the main agree with ours. For example, Stivelman, Hennell and Golembe³³ state that "Graham and Bell touched the heart of the subject . . . In the presence of a flexible mediastinum, the intrathoracic equilibrium in pneumothorax is very delicately adjusted, and any disturbance in the intrathoracic pressure on the treated side will have a proportionate effect on the intrathoracic pressure on the untreated side." Lenhart,³⁴ in experiments on rabbits,

found that unilateral open pneumothorax causes an intense dyspnea, provided the anesthesia is not too deep, and death, if unrelieved by closure of the thoracic opening. The volume of air respired per minute is decreased despite the increased respiratory effort; the excretion of carbon dioxide is impaired, often to a great degree; the respiratory quotient is constantly reduced; there is an increase in the total carbon dioxide content of the blood, and at the same time an increase in the hydrogen ion concentration. Finally he states that his opinion concerning the alteration of pleural pressures on both sides as a result of a unilateral open pneumothorax is in agreement with ours. Simon³⁵ has also come to practically the same conclusion as a result of his experiments.

In the discussion here of the effects of pneumothorax I have dealt particularly with the effects on respiration. But there are also other effects, for example, on the circulation. Sauerbruch summarized these as follows: "In pneumothorax the aspiration of the heart fails; a stasis results in the venous system. Measurements of the venous pressure in the femoral vein give in fact an increase of the pressure." As a result of asphyxia there is of course a rise in the arterial blood pressure; this is a general result of asphyxia and has no particular relationship to the question of asphyxia produced by pneumothorax. Yates has called attention to the importance of the pulmonary circulation in the inflation of the lung. He states that "Wherever intrapulmonary pressure exceeds intravascular tension a proportion of the blood destined under normal conditions to reach the compressed lung area is immediately diverted to adjacent channels offering less resistance. The capillary network that surrounds the air cells spreads, as its component vascular tubules are thus elongated by overfilling. If air passages are free and lung tissue is elastic, the result is increased inflation in the portion of lung to which extra blood has been delivered, a compensatory physiologic emphysema. On the contrary a temporary stoppage of pulmonary circulation produces atelectasis; permanent stoppage causes atrophy in the lung affected. In short, the pulmonary

circulation is the most important factor influencing pulmonary inflation, and fluctuation in pulmonary inflations accompanied by variations in the resistance to blood flow in the pulmonary circulation." These are important considerations. They are, however, secondary results of the alteration of pressure produced by the pneumothorax. Moreover, so far as the atelectasis resulting from a temporary stoppage of circulation is concerned, time is necessary for its accomplishment. The instantaneous effects on respiration which follow the creation of an open pneumothorax and the instantaneous improvement in the dyspnea which follows the closure of the opening must be explainable on the basis of the immediate pressure effects exerted on the lung parenchyma rather than on the more slowly developing effects of disturbances of the lung circulation. The ideas of Yates, however, are of great interest in connection with the mechanism of the production of compensatory emphysema. He himself tries to explain Skodaic resonance on the basis of an increased blood supply and therefore, as he reasons, an increase in the size of the alveoli, in the part of the lung which shows the hyperresonance. When this idea is put to the test of experiment, however, it is found that distention of the capillaries induces a diminution rather than an increase in the size of the alveoli. Experiments were carried out by H. J. Davis and myself³⁰ on a series of dog's lungs. Immediately after death from ether the chest was opened and the lower lobe of each lung was removed. One of the principal blood vessels to the lobe was then injected with physiological sodium chloride solution, in some cases an artery and in other cases a vein. A tight ligature was then placed around the hilus of the lobe, including both the vessel and the bronchus, and the lobe was quickly immersed in 10 per cent. formalin. In every instance the alveoli of the lobe whose capillaries were distended were much smaller than those of the other lung. This was found true not only for normal lungs but also for those with pneumonia. It seems probable therefore that Skoda's resonance in pneumonia cannot be explained on the basis of an increased blood supply to the part. The accompanying illustra-

tions (Figs. 6 and 9 inclusive) show that the size of the alveoli is diminished as a result of the distention of the capillaries. Conversely it might be expected that when the aveoli are greatly distended the capillaries are diminished in size. That this result actually happens has been shown by the interesting work of Chillingworth and Hopkins³⁷ who studied the question by placing dogs in a specially devised body plethysmograph which included the entire dog, with a tracheal cannula projecting outside. These investigators found that when the lungs are artificially distended by diminishing the plethysmographic pressure various results may occur as follows: (1) With slight distention there is a slight rise in the carotid blood pressure due to the freer passage of blood to the left heart. (2) With moderate distention a fall in carotid pressure occurs. (3) With full distention the carotid pressure falls approximately to zero. (4) With overdistention, causing emphysema and pneumothorax, there is less of a fall of carotid pressure than that produced by less distention. (5) The fall in the carotid blood pressure caused by moderately diminished plethysmographic pressure is due to failure in the supply of blood to the left heart, a failure which is due to pressure exerted almost directly upon the small pulmonary vessels.

Other effects of an open pneumothorax are marked loss of heat and the great danger of infection. Sauerbruch found that in unanesthetized rabbits in which he had established an open pneumothorax at a room temperature of from 20° to 22° C., the body temperature sometimes dropped as much as 3.5° C. within forty-five minutes. In dogs, also unanesthetized, within a half-hour he observed the body temperature fall 2° C. after making a pleural opening and rise again 1.6° C. within an hour after closing the opening. He also made the very striking observation that the heat loss in open pneumothorax is greater than in an extensive laparotomy with eventration of the intestine for the same length of time. For example, a dog's temperature fell only 1° C. after having his intestine lying out of his abdomen for forty-five minutes, and a rabbit subjected to the same experiment showed a heat loss of only 1.3° C. after forty-five minutes.

FIG. 6.

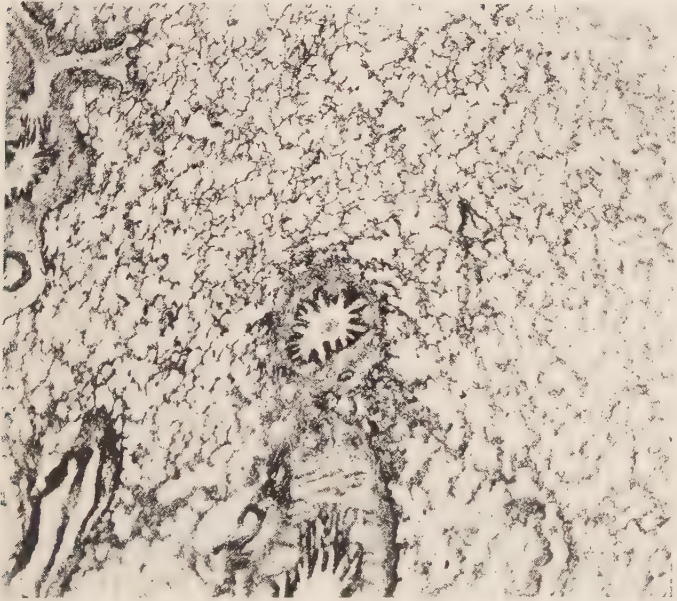
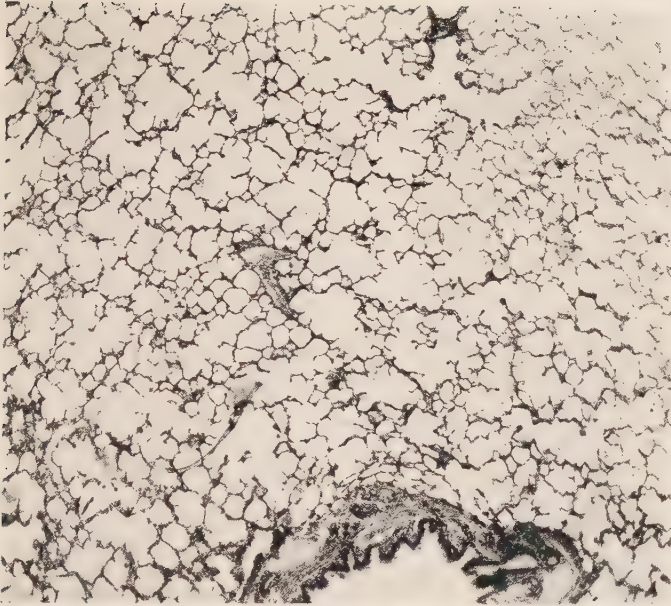


FIG. 7.

FIG. 6.—Normal dog's lung. Low power magnification.

FIG. 7.—Lung from same dog after injection of pulmonary vein with 0.85 NaCl solution. It is plainly evident that the alveoli have become smaller instead of larger. Even the bronchi are compressed. Skoda's resonance can therefore probably not be explained on the basis merely of increased blood supply to the part. Magnification the same as in the preceding figure. See text.

FIG. 8.

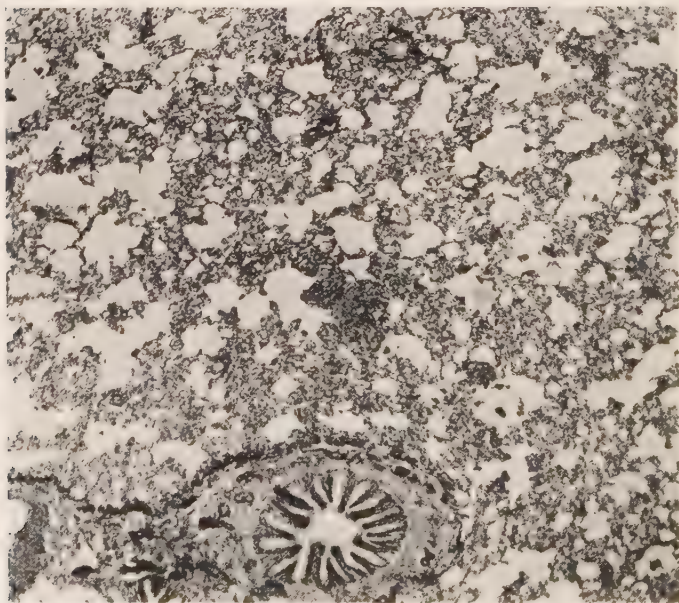
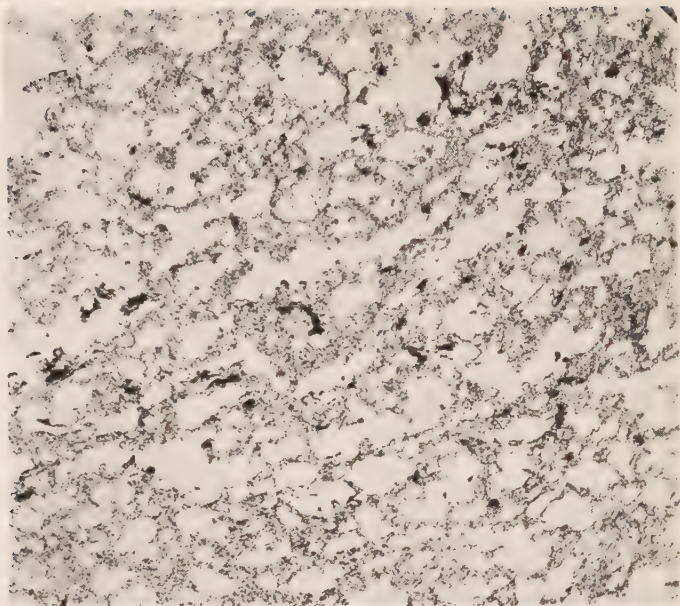


FIG. 9.

FIG. 8.—Pneumonic dog's lung. Low power magnification.

FIG. 9.—The same lung after injection of the pulmonary vein showing that also in the pneumonic lung, as well as in the normal lung, compression of the alveoli might be expected to follow an increased blood supply to the part. Same magnification as above. See text.

The danger of a general infection of the pleura in open pneumothorax is very great and is much greater than is the danger of a general infection of the peritoneum in the case of an open wound of the abdomen. There are probably two reasons for this fact; one is that with the suction of air through the pleural opening large numbers of bacteria are drawn in, and the other is that, with the lung retracted away from the chest wall, there is not the same possibility of sealing the opening as is the case in the abdomen by the action of the omentum and the viscera.

With reference to this discussion on the effects of pneumothorax the question naturally arises. Is positive pressure necessary in the performance of intrathoracic surgical operations? In cases without adhesions or without induration of the mediastinum it will be possible to make a large pleural incision in an individual whose vital capacity is high, particularly if those measures are applied which have already been discussed, namely, the delivery of the lung out of the incision, the insertion of gauze packs into the wound, etc., in other words, the use of any plan which minimizes the size of the actual opening. On the other hand, in patients with low vital capacities and without adhesions, the use of a large pleural incision invites disaster unless the means are at hand to institute some degree of positive pressure. In any case, it seems to me, an intrathoracic operation should not be undertaken unless one is prepared to administer positive pressure. No cumbersome apparatus is necessary for this. An ordinary nitrous-oxide machine with a tightly fitting face piece, as suggested in 1910 by Bunnell,³⁸ is all that is necessary. Such a device has the additional advantage that not only can positive pressure be applied but that also oxygen can readily be supplied quickly in case of the development of a serious anoxemia. In any case, relative speed in operating is highly desirable. Not only does a quick operation diminish the chance of a serious anoxemia but it also minimizes the loss of heat through the incision, a point which is very important in thoracic work.

It is now well known that the air in a closed pneumothorax

disappears completely within a few weeks and sometimes within a few days. The early pioneers in the therapeutic use of artificial pneumothorax, as for example Forlanini, thought it would be advisable to use nitrogen because supposedly it would disappear less rapidly. Later work, however, showed that, owing probably to the diffusion of gas from the lung into the pleural cavity and *vice versa*, the gas in a pneumothorax, even if pure nitrogen originally, soon came to have the composition of alveolar air. For that reason it is now customary to use air for artificial pneumothorax rather than nitrogen because of its greater convenience. This transfer of gases back and forth between the lung and the pleura is analogous to a process of respiration outside of the lung. It resembles therefore somewhat the respiration in the alimentary canal which was studied by Woodyatt and myself³⁹ in 1912. Despite the interest attached to the phenomenon of the transfer of gases from the lung into the pleural cavity and its reverse, it has never received the attention which it deserves. The diffusion of gas into the pleural cavity is so rapid after death that, at least in the case of the dog, an easily demonstrable bilateral pneumothorax may be seen in X-ray plates taken one hour after death. In one instance, from one pleural cavity of a 10 kilo dog 32 c.c. of gas were obtained twenty-four hours after death, an analysis of which by Dr. P. A. Shaffer showed 17.5 per cent. of CO₂. (See Figs. 10 and 11).

Another interesting problem dealing with alterations of the intrapleural pressure concerns the question of the formation of pleural exudates. The rapidity with which fluid accumulates in cases of infection of the pleura with the hemolytic streptococcus and the fact that dyspnea is often a prominent feature of streptococcal pneumonia suggested the idea that fluid might actually be sucked out of a very edematous pleura by the negative pressure during the forced inspirations. In this type of pneumonia, as is well known, the dyspnea is often so marked that maximal inspiratory efforts are required in order to take in enough air to maintain life, due largely to an actual obstruction of the air passages with the products of inflammation.



FIG. 10.—Spontaneous bilateral pneumothorax in a normal dog one hour after death from ether. Much of this gas in the pleural cavity is CO_2 which has apparently diffused out of the lung into the pleural cavity after death. See text.



Fig. 11.—Another normal dog showing a spontaneous bilateral pneumothorax one hour after death from ether.

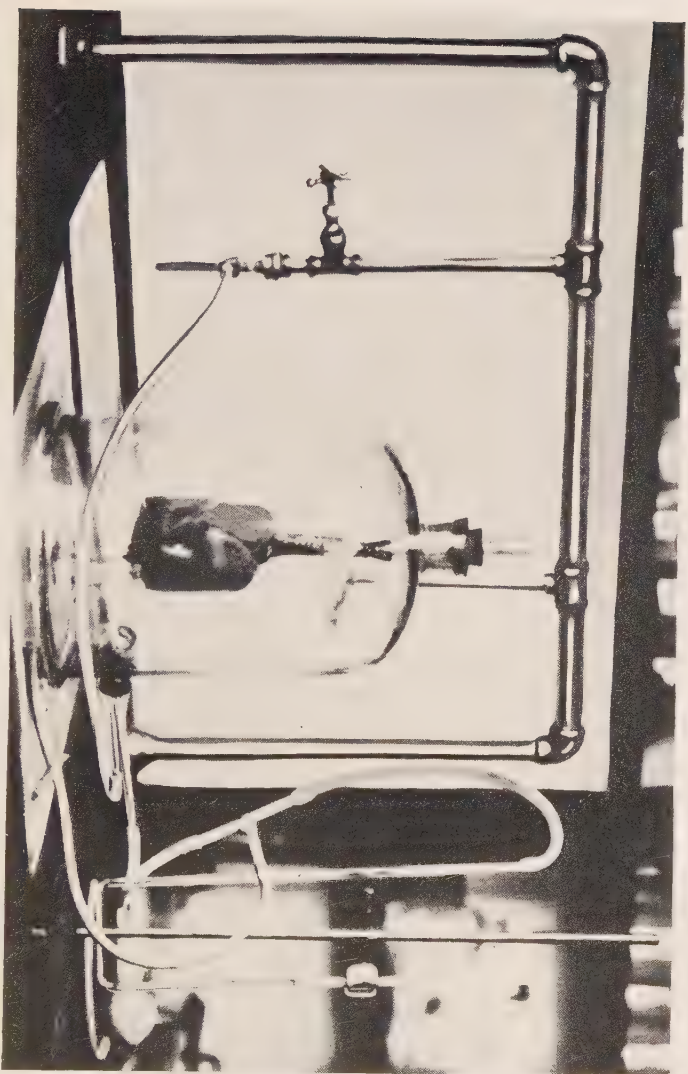


Fig. 12.—Apparatus used for the determination of the effect of intrapleural pressures in the production of pleural exudates.
See text.

The conditions therefore are ideal for the production of a very low or a markedly negative pressure in the pleural cavity. It would seem reasonable to expect that under such conditions fluid might be sucked out of a "wet" pleura in a manner analogous to the aspiration of fluid out of any very edematous tissue by means of an aspirating syringe. With these ideas in mind the problem was put to an experimental test.⁴⁰ An artificial thorax was constructed by means of a bell-jar and a pair of lungs. When the air in the bell-jar around the lungs was aspirated, the lungs would inflate in a manner similar to an ordinary inspiration. When edematous lungs were used, either those from a case of pneumonia in the human being, or dogs' lungs made edematous by pouring into the trachea water containing a little dilute acetic acid, pleural exudate could be seen to pour from the lung surface with each imitated act of respiration. Contrary to the expectation, however, the fluid poured out more rapidly during the act of expiration than during inspiration. The explanation seemed to be that during inspiration the pleura became saturated, and then with the sudden decrease of surface produced by the act of expiration, the fluid was literally squeezed out. So far as I know, this is the only attempt that has been made to study the actual mechanism of the production of pleural exudates. The problem needs more study. (Fig. 12).

As was stated above on page 127 a resumption of the study in the human of the effects produced in the opposite pleural cavity by injections of air into one showed that when relatively great pressures are reached on one side the response on the other side agrees less closely than is the case when one is dealing with small pressures. Dr. Duff S. Allen and I have recently reinvestigated this question on five human cadavers with normal thoraces. The experiments were carried out immediately after death, while the bodies were still warm and before *rigor mortis* had occurred. Each of two cannulæ of the same size was inserted through the sixth interspace into each pleural cavity. These were connected with water manometers and also, through a side connection, with an atomizer bulb. By blowing air into one pleural

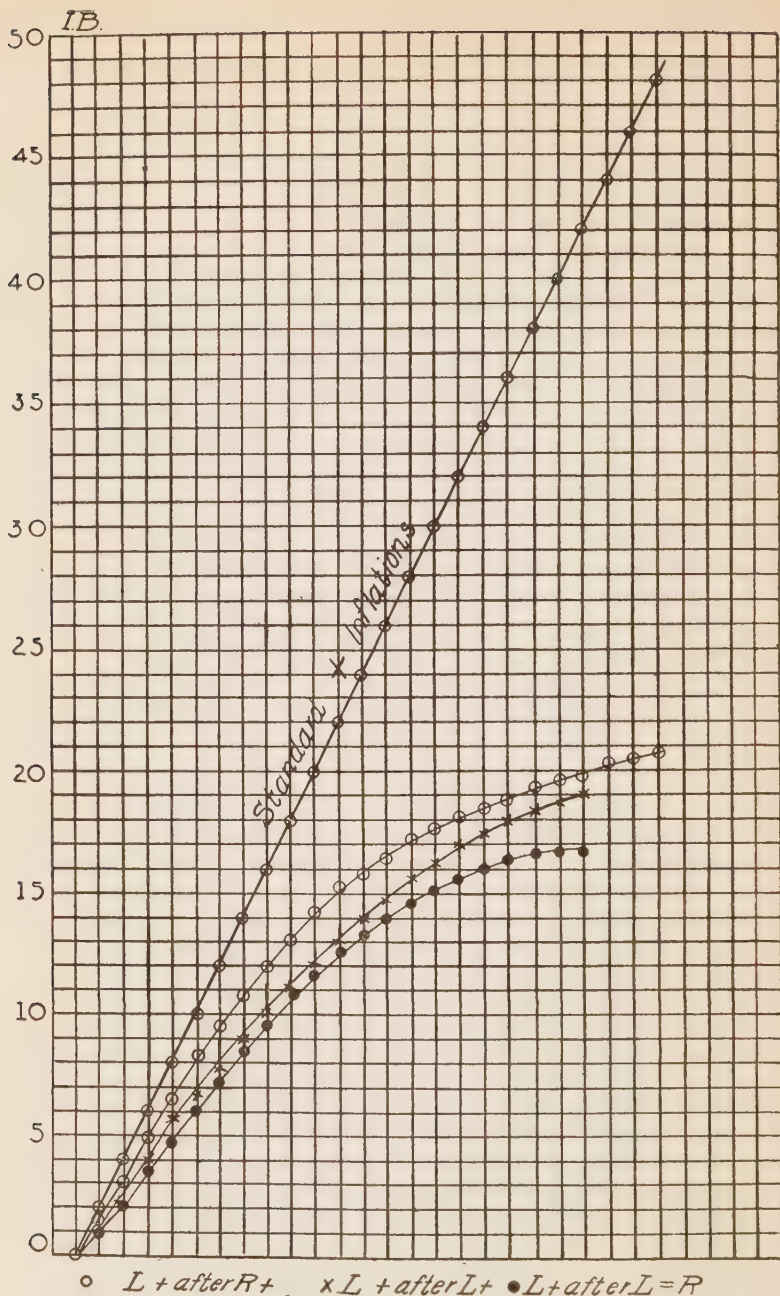


FIG. 13.—Curves of intrapleural pressure in the human. The numbers at the left represent centimeters of water pressure. Comparison should be made between the diagonal line ("standard + inflations") and the curves. It will be noted that all the curves tend to run parallel with the diagonal when a pressure below 10 cm. is used. This indicates that, below and up to an alteration of pressure in one pleural cavity equal to 10 cm. of water, the pressure in the other is nearly equal to it. With greater pressures, however, the agreement between the two sides is less close. See text.

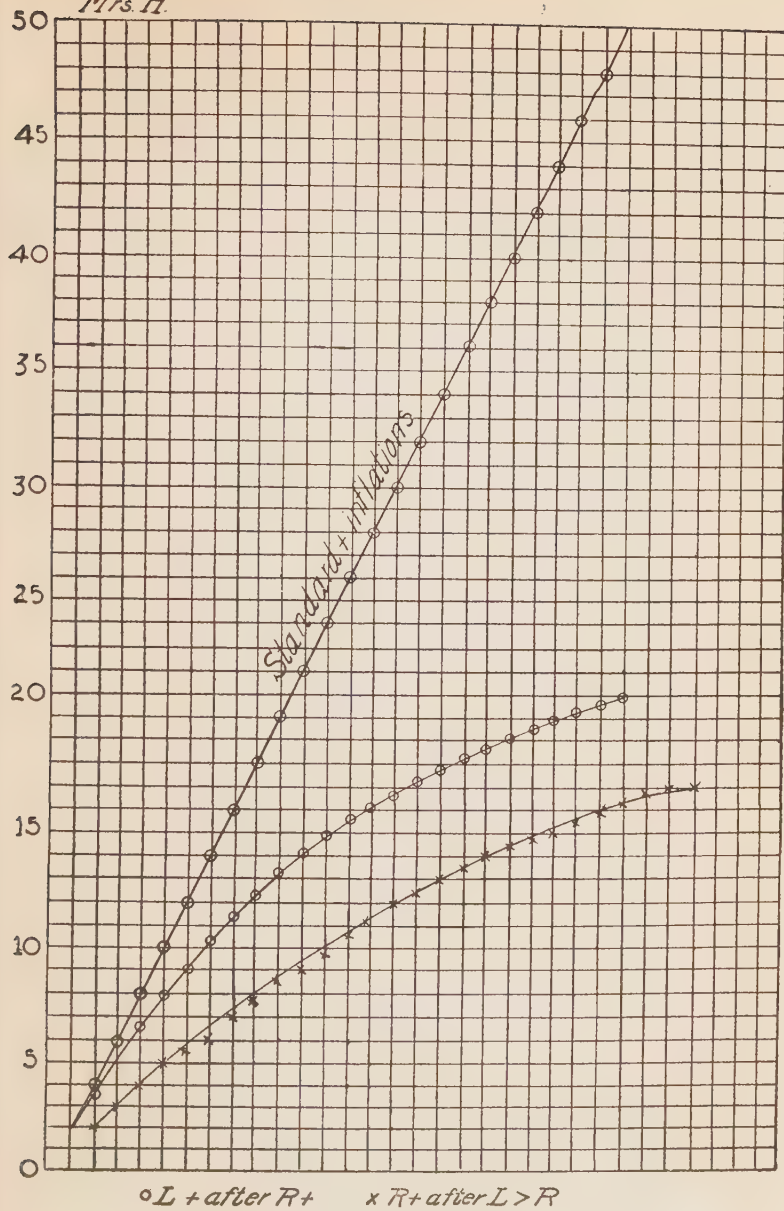


FIG. 14.—Another experiment similar to that shown in Figure 13.

cavity the pressures on both sides could be read simultaneously. It was observed that the pressures in the two pleural cavities agreed most closely when a positive pressure was first created on one side followed in two or three minutes by the injection of air into the opposite side. For example, if a small positive pressure were first created by the injection of air into the right side and then if the left side were gradually inflated by increasing amounts of air, the pressures in the two pleural cavities would agree more closely than when the experiment was carried out in any other way. In fact when dealing with pressures up to about 15 cms. of water the agreement is rather close as may be seen by reference to the accompanying diagram. (Fig. 13). The curve of results obtained in the above manner is designated "L+ after R+"⁴¹ If, however, the pressures in the two pleu-

2	1.5	26	16.5
4	3.0	28	17.25
6	5.0	30	17.50
8	6.5	32	18.0
10	8.5	34	18.5
12	9.5	36	18.75
14	11.0	38	19.25
16	12.0	40	19.75
18	13.0	42	20.0
20	14.5	44	20.25
22	15.25	46	20.50
24	16.0	48	20.75

ral cavities were equal at the start, then continued inflation of one side was not followed by pressure readings in the opposite side of such close agreement, as shown in the curve designated "L+ after L=R." The middle one of the three curves, designated "L+ after L+" in figure 13, shows the results obtained when a positive pressure was first created in the left pleural cavity followed by inflation of that cavity in the usual way. The differences obtained in accordance with the actual methods of conducting the experiment probably account for some of the discrepancies and disagreements reported by various observers. Time will not permit a discussion here of the various possible explanations of the differences which suggest themselves.

In this general summary of the effects of alterations of intrapleural pressure which has been given above, it is recognized that some of the ideas and theories here set forth may perhaps have to be modified from time to time as later work sheds new light on the subject. At the present time, however, it seems to me that no general theory of the action of pneumothorax explains all the known facts so well as the one which has been advanced above. In principle the theory is the same as that expressed in 1918; it differs only in relatively unimportant details. Those who have disagreed with the principles of this theory have invariably failed to offer a constructive alternative hypothesis which is at all in accord with all the known facts concerning the action of pneumothorax.

BIBLIOGRAPHY

² Quoted from H. G. Wells: "The outline of History," 1920, Vol. i, p. 403, the Macmillan Company, New York.

³ Quoted by Heynsius: Arch. f. d. ges. Physiol., 1882, xxix, 267.

⁴ Die Mechanik und Therapie des Pneumothorax, 1902, quoted by Emerson in "Pneumothorax," Johns Hopkins Hospital Rep., 1903, xi, 194.

⁵ Pneumothorax, Johns Hopkins Hospital Rep., 1903, xi, 1.

⁶ Emerson: *loc. cit.*

⁷ Sauerbruch: Zur Pathologie des offenen Pneumothorax und die Grundlagen seines Verfahrens zu seiner Ausschaltung, Mitteil. a. d. Grenzgeb. d. Med. u. Chir., 1904, xiii, 399.

⁸ Mayer, L.: Les Bases Physiologiques de la chirurgie Pleuropulmonaire, Ann. d. I. Soc. Roy. d. Sciences Med. et Nat. de Bruxelles, 1906, xv, I.

⁹ Hewson, quoted by Emerson: *loc. cit.*, Footnote 1, pp. 11-13.

¹⁰ Cruveilheir: de l'empyème et de quelques expériences sur les animaux, Bull. de l'Acad. de méd., 1836, i, p. 280.

¹¹ Graham, E. A., and Bell, R. D.: Open Pneumothorax: Its Relation to the Treatment of Empyema, Am. Jour. Med. Sci., 1918, clvi, 839.

¹² This was later modified somewhat in another article, Graham, E. A.: The Importance of the Vital Capacity in Thoracic Surgery, Jour. A. M. A., because the average vital capacity as originally stated was too low.

¹³ Matas, R.: The value of Artificial Aids to Respiration in Acute Operative Collapse of the Lungs, Arch. Surg., 1922, v, 110.

¹⁴ Duval, P.: Les donnees actuelles de la chirurgie intrathoracique unilaterale en plevre libre, Presse med., 1922, xxx, 409.

¹⁵ Yates, J. L.: Effects of Acute and Chronic Pneumothorax: A preliminary Report, *Am. J. M. Sci.*, 1923, clxv, 1.

¹⁶ Snyder, J. W.: Studies in Intrapleural Tension, *Arch. of Surg.*, 1924, viii, 364.

¹⁷ Duval, P.: Les plevres communiquent-elles normalement chez le chien, *Presse med.*, 1923, No. 68, p. 733.

¹⁸ Matas, R.: Remarks on the so-called Mediastinal Septum of the Dog, in relation to the Pneumothorax Problem in Man, *Arch. of Surg.*, 1924, viii, 336.

¹⁹ Garland, G. M.: *Pneumodynamics*, New York, Hurd and Houghton, 1878, p. 53.

²⁰ Calvert, J.: On the Intrapleural Pressure and Manner of Contraction of the Lung in Pleural Effusion, *St. Bartholomew's Hosp. Rep.*, 1892, xxviii, 131.

²¹ Graham, E. A.: A Reconsideration of the Question of the Effects of an Open Pneumothorax, *Arch. Surg.*, 1924, viii, 345.

²² Graham, E. A.: Principes qui decoulent de la chirurgie intrathoracique, *Presse med.*, 1923, xxxi, (February 14).

²³ Haldane, J. S., and Poulton, E. P.: The Effects of Want of Oxygen on Respiration, *Jour. Physiol.*, 1908, xxxvii, p. 390.

²⁴ Haldane, J. S., Meakins, J. C., and Priestley, J. G.: The Effect of Shallow Breathing, *Jour. Physiol.*, 1919, lii, p. 433.

²⁵ Dautrebande and Spehl: Les causes de la dyspnee et de la mort dans le pneumothorax ouvert, *Bull. de l'Acad. Roy. de Med. de Belgique*, 1922, Nov. ii, p. 1.

²⁶ Lundsgaard, C., and Van Slyke, D. D.: Cyanosis, *Medicine*, 1923, ii, p. 1.

²⁷ Sackur: Zur Lehre vom Pneumothorax, *Ztschr. f. klin. Med.*, 1896, xxix, 25.

²⁸ Graham, E. A.: Some Surgical Aspects of Asphyxia, *Ve Congres de la Societe internationale de chirurgie*, Paris, 1920.

²⁹ Graham, E. A.: Recent Phases of Thoracic Surgery, *Jour. A. M. A.*, 1923, lxxx, 1825.

³⁰ See: Review of War Surgery and Medicine, Office of the Surgeon-General, Washington, 1918, No. 4, 1-27.

³¹ Stone, W.: Management of Postpneumonic Empyema Based upon 310 Cases, *Am. Jour. Med. Sci.*, 1919, elviii, 1.

³² Holt: The Siphon Treatment of Empyema in Infants, *Am. Med.*, 1913, xix, 381.

³³ Stivelman, B. P., Hennell, H., and Golembe, H.: Clinical Significance of Altered Intrathoracic Equilibrium in Pneumothorax, *J. A. M. A.*, 1922, lxxviii, 1450.

²⁴ Lenhart, C. H.: Open Pneumothorax: An Experimental Study of the Functional Pathology of Sucking Chest Wounds, *Arch. Surg.*, 1920, i, 336.

²⁵ Simon, S.: Effect of Artificial Pneumothorax on Collateral Lung, *Am. Rev. Tuberc.*, 1921, v, 620.

²⁶ Davis, H. J., and Graham, E. A.: The Effect of Pulmonary Capillary Distention upon the Size of the Alveoli, not yet published.

²⁷ Chillingworth, F. P., and Hopkins, R.: Physiologic Changes Produced by Variations in Lung Distention, ii. Efficiency of the Pulmonary Circulation in Overcoming Obstruction, *Am. Jour. Physiol.*, 1920, li, p. 289.

²⁸ Bunnell, S.: The Use of Nitrous Oxide and Oxygen to Maintain Anesthesia and Positive Pressure for Thoracic Surgery, *California State Jour. of Med.*, 1910, Jan. Also see Bunnell, S.: Positive Pressure for Thoracic Surgery with Nitrous Oxid-oxygen Anesthesia, *Am. Jour. of Surg.*, 1923, Oct., pp. 98-105.

²⁹ Woodyatt, R. T., and Graham, E. A.: Alimentary respiration: The secretion of carbon dioxide by the alimentary mucosa and its relation to eructations of gas and abnormal inflation of the stomach and intestines, *Trans. Chicago Path. Soc.*, 1912, viii, 354.

³⁰ Graham, E. A.: Influence of Respiratory Movements on the Formation of Pleural Exudates, *Jour. A. M. A.*, 1921, lxxvi, 784.

³¹ Under such conditions the numerical values, for example, were found as given in the column below. The close agreement between the pressures of the two sides up to 10 cm. of water is in harmony with our results published in 1918.

PHYSIOLOGICAL, CHEMICAL AND CLINICAL STUDIES ON PITUITARY PRINCIPLES *

DR. JOHN J. ABEL

Professor of Pharmacology, Johns Hopkins University

THE designation *Hypophysis cerebri* for the organ concerning which I am to address you this evening is to be preferred to the more ancient one, *Glandula pituitaria cerebri*, for the reason that the former involves no other idea than that of location in reference to the cerebrum, while the qualifying adjective, *pituitaria*, of the second designation connotes a secretory function and definitely commits one to a theory of external secretion of the organ. The word *pituita*, as used by Cicero and other writers of the classical period of Roman literature, means mucus, slime or phlegm and our predecessors of an earlier day from Galen to Vesalius developed the theory that the brain voids mucus and excrementitious matter through the infundibular stalk into the body of the gland from which these filter down in some way into the nose and upper pharynx. The term *colatorium*, or strainer, was frequently applied to the gland and we are told by Hyrtl that the word *sentina* was also used in this connection. Now this latter term, like *pituita*, was in good use in the classical period and was applied to the filthy water that collects at the bottom of a ship—the bilge water, and was furthermore used to designate the lowest of the people, the dregs, refuse or rabble of a city or state. I have ventured to call your attention to these words and their meaning because it is plain that the writers of Galen's days (131–200 A.D.) had committed themselves to this definite theory of the function of the hypophysis. To them it was very obviously an organ of external secretion with whose help the brain got rid of excrementitious matter much as the liver secretes bile into the digestive tract. I have not made any

* Delivered April 12, 1924.

extended historical study of earlier writings on this subject and cannot state how long this theory that the hypophysis is a detergent organ of the brain persisted. Cushing has called attention to the fact that Richard Lower published a dissertation in 1672 (*Dissertatio de Origine Catarrhi*) in which he seems to have been the first to disprove the Galenic theory of pituitary function. "For whatever serum is separated into the ventricles of the brain and issues out of them through the infundibulum of the *Glandula pituitaria* distills not upon the palate but is poured again into the blood and mixed with it." It must however have been believed in until close to the middle of the 18th century if not later. Only the other day I came upon the theory in looking up a point in the excellent treatise of Nicholas Monardes, a small book in which that very judicial-minded Spanish physician describes the remarkable new drugs that had been brought to Europe after the discovery of the new world, their physical properties, mode of administration and especially their therapeutic or curative powers. Monardes is writing a little later than the middle of the 16th Century, at the close of the lifetime of Andreas Vesalius. The first edition of this book appeared in 1565, a second in 1569 and a third in 1571. The second edition was translated in 1569 by the distinguished botanist C. Clusius into Latin and there it is stated that one of the many remarkable attributes of a pill made by chewing tobacco leaves with burnt lime from mussel shells is to conduct away the slimy juices of the brain which, passing into the stomach, are later digested! There is no specific mention of the hypophysis in this statement of Monardes as he evidently assumes that his reader is familiar with the mechanism by which slimy juices pass from the brain into the stomach.

I have descanted for a few moments on these ancient terms, *pituita*, *colatorium* and *sentina*, and the definite theory of external secretion which originated their employment, because it may be safely asserted, I think, that a century hence when our successors read the literature of our day in respect to the endocrine organs, they will have fully as much ground for amusement or for criti-

cism as we have when we read that the function of the pituitary gland in the human being is that of affording an outlet for the slimy juice of the brain. When it comes to some of the lower animals, as the myxinoid fishes in which the pituitary tube opens on the roof of the head, where its external opening serves as an external nostril for the olfactory organ, the idea of a slime-excreting organ may not be so fanciful after all. Also bear in mind that we possess a pharyngeal hypophysis (5 mm. long) which is regarded as an active functioning gland, and that there exists a *canalis cranio pharyngeus* which is generally closed but in which, though very rarely, the entire hypophysis may be lodged. According to Gaskell's theory, indeed, the infundibulum or stalk of the posterior part of the pituitary body represents an ancestral mouth to which the ventricles of the brain and the central canal of the cord acted as the stomach and intestine. To cite his own words, "I have described how my investigations into the Vertebrate nervous system have led me to the conclusion that that system is composed of nervous material grouped around a central tube which was originally the alimentary canal of the Invertebrate from which the Vertebrate arose . . . I have purposely omitted the consideration of the pituitary body because it does not in reality belong to the central nervous system, it represents, in my opinion, the Crustacean green glands, and will be considered when I come to deal with the foundation of the Vertebrate skeletal tissues, and of the excretory organs."

Bremer, a recent Belgian reviewer of our present knowledge of the physiology of the hypophysis, prefaces his article with this statement: "The significance of the hypophysis remains a mystery, but hypotheses and theories abound and there is a singular contrast between the abundance and assurance of the current theories in respect to the functions of the gland and the rarity and incertitude of established facts." Percival Bailey, who wrote an admirable paper entitled *Die Function der Hypophysis cerebri* in *Ergebnisse der Physiologie* for 1922, but who could not take into account any work published later than

December 1921, also comments on the unsatisfactory character of the experimental basis of the theories which are now being exploited by certain over-enthusiastic clinicians.

One is almost tempted to say of the hypophysis what Fontani said of the brain more than one hundred and ninety years ago: "obscure as to its texture (structure), more obscure as to its diseases and most obscure of all as to its functions" ("obscura textura, obscuriores morbi, functiones obscurissimæ").* The state of affairs is after all not quite so bad as this; I would not have you look upon me as a pessimist in this field, as one who is inclined to belittle our knowledge in respect to the organs of internal secretion; quite the contrary. May I not digress for a moment, in order to clear myself of the possible charge of under-rating the importance of endocrinology? One has only to turn back to the textbooks and medical encyclopedias of 40 years ago to realize the extraordinary importance of the great number of actual facts at our disposal to-day. The very notable discovery of Addison in 1855 in respect to the constitutional and local effects of disease of the suprarenal capsules and the significant bio-chemical observation of Vulpian in 1856 that the fluid expressed from the interior of these capsules turns green on the addition of ferric chloride, like the still earlier very remarkable experimental physiological work of Berthold in 1849, which last proved conclusively that the testes (and the ovaries by inference) have an internal secretion which is responsible for the secondary sex characteristics, did not at once arouse investigators to take up work in endocrinology, all along the line, as on looking back on that period would have thought to be the case. Even the great discoveries of Claude Bernard (1857) in reference to glycogen and his definite assertion that the liver is to be regarded as an organ of internal secretion (although this phrase according to Gley was first used by Charles Robin in the sixties and by Paul Bert a little later) because it stores carbohydrates in the form of glycogen and at need converts this into blood sugar which passes into the blood stream, did not cause

* Quoted from Hyrth. I have been unable to consult the original.

that rush into the endocrine gold fields which started in the later '80s of the last century. This stampede may be said to have been due to a number of discoveries all of which took place within a few years of each other and during my own apprenticeship as a researcher. It is an established fact that the earliest discoveries of Addison in 1855, of Vulpian in 1856, of Bernard in 1857, of Berthold in 1849, of Basedow in 1840 and of Graves in 1835, of Brown-Séquard in 1856 and of Schiff in 1859-1866 did not either individually or collectively by their own importance, great as this was in each instance, start that attack on these problems of endocrinology on a wide front which is so marked a feature of present-day science. More was required and one of the most stimulating of the newer discoveries was that of replacement therapy. The events I refer to are the following:

- (1) Marie's announcement in 1886 of his discovery of a "peculiar non-congenital hypertrophy of the upper, lower and cephalic extremities," a condition now known as acromegaly, and the suggestion which was thrown out a year later (1887) by Minkowski that the hypophysis plays a rôle in this syndrome.
- (2) The discovery of Sandström (1880) that in human beings and mammals certain hempseed-sized glands are situated in or in close proximity to the thyroid glands, and the experimental demonstration of Gley in 1890-1891 that these apparently insignificant structures stand in a peculiar and causal relationship to tetany.
- (3) The theory of Moebius announced by him in 1887 that Graves' or Basedow's disease is due to an abnormally increased activity—a hyperfunctioning of the thyroid gland.
- (4) The demonstration of Murray in 1891 that the myxedema described by Gull in 1872 and studied by Ord (1878), Hadden (1882) and others could be greatly alleviated or even cured by the administration of thyroid extract. Swiss surgeons, the two Reverdins and Kocher, had previously found (1882-3) that total thyroidectomy is followed by a post-operative condition very similar to this spontaneous myxedema of the English physicians.
- (5) The demonstration in 1889 by von Mering and Minkowski that complete removal of the pancreas from dogs was followed

by a state of disease which resembled in practically all respects severe diabetes mellitus in man. That very notable discovery was immediately followed by experimental work in the same field by Hédon (1892), by Lépine and others, and was the forerunner of all those extraordinary discoveries in regard to the pancreas that have now culminated in the preparation and practical use of insulin by Banting, Best and Macleod and their collaborators. (6) Brown-Séquard's positive statement in 1889 that the subcutaneous injection of testicular extracts into human beings exercises a marked tonic influence, more particularly in elderly individuals, as was claimed by him to have been demonstrated in his own case, must also be recalled here. For while this work of Brown-Séquard was far from convincing it nevertheless excited universal attention. In the opinion of his compatriot Gley "it has proved to be of epoch-making significance."

It will be seen that the six significant events which I here enumerated all fall within the years of 1887 to 1891, *at least it may be stated that the particular part of each of these events which is most significant in regard to its effect on the study of the endocrine organs falls in this short space of four years.* It was only in the later eighties, as many of us can testify who walked the wards at that time, that the more advanced of our clinical instructors began to call the attention of their students to some of the new marvels. I can recall as if it were yesterday the first case of myxedema which I had the opportunity to study when I was a graduate student of medicine in Nothnagel's clinic in Vienna in the autumn and winter of 1889-90. Little, I think, did some of our teachers of that day foresee that these examples of disease which were regarded more or less as interesting rarities were to open up a new world to investigators in the biological and medical sciences.

Immediately, in the early nineties, chemists and physiologists and pathologists all began to be very active in this field and endocrinology began that advance which has continued to this day and will no doubt continue to hold the attention of students for centuries to come. The thyroid and suprarenal glands were

successfully attached on the chemical side in the nineties. Physiologists, as Schafer and Oliver, Cybulski, Howell, Szymonowicz and many others, found that extracts of various endocrine glands, as the adrenals and the hypophysis, intravenously administered, exerted the most surprising effects on the cardiovascular and other structures of the body. And how extraordinary has been the course of discovery since the early nineties of the last century! Secretin, Insulin, Thyroxin, Epinehrine, Pituitrin and other names of endocrine principles come to mind as indicating the progress that has been made possible by the conjoint labor of clinicians, physiologists, pharmacologists, chemists, pathologists and biologists. We now speak with confidence and familiarity of hormones, growth principles, vitamins and the like, substances which in very minute amounts act as chemical or humoral integrating agents, or as stimulating or sensitizing agents for particular structures of the body. We speak of the interactions and interdependence of many of the endocrine organs although we are here dealing with most intricate and involved mechanisms. New evidence is however coming to hand almost daily in support of the thesis that an interrelationship exists between some, at least, of the most important of the endocrine organs. Much of this new evidence is being furnished by the younger school of experimental zoologists here and abroad.

It may be questioned if there is a single branch of the medical art that has remained uninfluenced by the discovery of the functions of endocrine organs. Only the other day my colleague, Professor Meyer, was commenting to me on the enormous significance of the abnormalities of one or more of the structures as affecting the behavior response of his patients toward their environment. At the time of our conversation an autopsy had been made upon a young woman, a homo-sexual pervert, who had committed suicide. The sexual organs, particularly the uterus, were very poorly developed and the suprarenal glands were among the smallest ever encountered in our autopsy rooms. The influence of the endocrine organs, more especially the gonads, on the psychic response of an individual to his environment

appears to be generally admitted. Their influence on our physical make up is also beyond question. The thyroid gland is especially important among these organs because of its extraordinary influence in intra-uterine life on both mind and body. Taking the two things together, that is to say, the influence of endocrine organs on both mental and somatic processes, it is not surprising that many see in the action and interaction of these organs, in their greater or lesser activity, in their influence on heredity, and in the assumed relations that subsist between some of them and the autonomic (sympathetic plus parasympathetic) nervous system, a full explanation for the differences in mind and body that exist between any two individuals taken at random from among the millions of any given race.

(At this point the lecturer showed two models of a recently removed human hypophysis, which were exact reproductions of both the size and natural appearance of the two main divisions of the gland. He also showed similar replicas of the bovine hypophysis, calling attention to the fact that the human gland weighs on the average only 0.612 gram (Erdheim) while the bovine gland weighs about 2.4 grams (Houssay), and that in both instances the neural or posterior lobe, with whose chemical composition and functional character this lecture is mainly concerned, constitutes only about one-sixth of the active hypophysis. Drawings were also exhibited in which the lesser divisions of the organ, the pars intermedia, pars tuberalis and infundibular stalk, were shown. The histological differences in the structure of the various divisions were briefly described. Since it was necessary to discuss certain recent findings of the lecturer in respect to the pharmacological properties of the tissues composing the floor of the third ventricle, anatomical drawings of the hypothalamus and the contiguous parts of the brain were shown and a brief account of our fragmentary knowledge of the physiological significance of this region of the brain was given.)

What are the chemical principles that have to be considered in the case of the hypophysis? Robertson has given the name tethelin to the growth principle of the anterior lobe and he has separated from this lobe a mixture of substances which he believes to contain this principle. This mixture, it is stated, produces striking effects on the metabolism and growth of experimental animals, but it must be borne in mind that thus far no chemical individual, whether it be given the name tethelin or

any other, has yet been isolated from this lobe, as is frankly admitted by Robertson. P. E. Smith of the University of California finds that Robertson's alcohol-soluble tethelin does not favor the growth of hypophysectomized tadpoles. Smith could get such tadpoles to grow only after feeding them the insoluble residues from which the tethelin had been removed by Robertson's methods. Robertson declares that the negative results which Smith has obtained by feeding hypophysectomized tadpoles with tethelin is due to the fact that he has added this principle in the form of a solution to the water in which the tadpoles exist and that the animals do not absorb the principle in sufficient concentration to get its physiological effect. It is evident therefore that we are encountering absolutely contradictory statements in reference to the physical and chemical properties of the growth principle which is undoubtedly present in the anterior lobe of the hypophysis in amphibia. And whether it is mainly found among the alcohol-soluble principles of the gland or remains in the less soluble residue appears for the time being to be undecided.

That a growth-maintaining principle of an unknown chemical nature is present in the anterior lobe of the hypophysis of amphibians has, however, been established with certainty. In addition to that of Smith, the work of Allen with tadpoles of *Rana pipiens*, that of Uhlenhuth with *Amblystoma* larvæ, and that of Huxley and Hogben with the Mexican salamander, *Amblystoma tigrinum*, has yielded results of the greatest value in this field.

Evans and his collaborators have recently demonstrated that characteristic effects upon growth, œstrum and ovulation can be induced in rats by the daily intraperitoneal injections of fresh anterior lobe substance. We have here further evidence of the existence of a specific hormone (or hormones) in the hypophysis. A further consideration of this newer work must be left to Professor Evans who is to follow me in a lecture in which he will no doubt give a full account of his own experiments with anterior lobe extracts, as well as of those of his predecessors.

The much smaller posterior lobe has been worked at very intensively during the past twelve years or more and we are

now getting much definite information in respect to its active principle. Physicians employ a simple extract which is furnished in the form of a sparkling clear solution in small sealed ampules that contain $\frac{1}{2}$ or 1 c.c. This simple watery extract, which contains all of the chemical substances present in the gland, except the proteins and certain lipoidal constituents, is of very great service in medicine, and especially in obstetrics where it rivals and often excels that old-time remedy, ergot. Unfortunately, this highly active pituitary extract is often wrongfully used and has a number of times caused the death of women when used by inexperienced physicians or midwives. If employed in overdose and at the wrong stage of labor, its action on the uterus may be so violent as to cause this organ to be ruptured. There are many other, both medical and surgical uses of this very potent drug. It has been found in recent years that it is the only known substance that will quickly control the excessive flow of urine in that distressing condition known as diabetes insipidus. As is well known to all of you, in diabetes insipidus the patient may void 20 or more liters of urine a day and is tormented by an excessive thirst. Naturally it is very difficult for such persons to go about in society and to attend to their business. But now if a little of a good pituitary extract should be injected under the skin of such patients, or if it should be dropped into the nostrils, the excessive flow of urine will be promptly diminished, the thirst will disappear and the individual will soon be made quite comfortable. The daily urinary output will go down to a very few liters or may reach the normal. The remedy may have to be given every 3 or 4 hours at first and later perhaps but once a day. The remedy may be continued as long as the disease persists. This drug removes the symptoms in almost all cases of diabetes insipidus and is therefore of the greatest service to those afflicted by this disease.

Now what chemical principles are we dealing with in an extract of the posterior lobe? Is there more than the one hormone present? The early efforts of Cyon, Houssay and Engeland and Kutscher at isolating an active substance or sub-

stances from the hypophysis are of historical interest only. An abstract of the work of these investigators is given by H. Fühner in the historical introduction to his paper (1913) entitled "Pharmacological Investigations on the Active Principles of the Hypophysis." Until very recently the idea that this lobe contains three or four or even more specific principles has been firmly maintained by several investigators. Eleven years ago the skilled chemists of the Farbwerke-Hoechst Company (see Fühner) announced that there are four crystalline principles present. These chemists claimed to have isolated these principles in the form of sulphates. Later, not on the basis of chemical isolation but on conclusions which were drawn from the use of certain solvents and from physiological reactions, Dale and Dudley assumed that there are three characteristic principles and possibly a fourth. My collaborators and I have found ourselves unable to agree with the views of the above named investigators. Our position may be briefly put as follows; and we believe that our experiments demonstrate the correctness of our conviction. We hold that the bovine posterior lobe (including the pars intermedia and the neural portion of the hypophyseal stalk) contains only one specific principle, or hormone. This principle has been isolated by us in the form of a tartrate for which we cannot as yet claim chemical purity and individuality, but which is of extraordinary potency as a smooth muscle-stimulant, being 1000 to 1250 times more powerful than the acid phosphate of histamine, a base which has hitherto held the ranking position in this respect. Continued studies on our part have shown that this tartrate displays all of the characteristic physiological properties of a good aqueous extract of the posterior lobe of the hypophysis. In addition to its action on the uterus, it causes a prolonged rise in the arterial pressure, with constriction of the peripheral arterioles and capillaries, a brief diuresis in the green-fed rabbit, has an antidiuretic action in diabetes insipidus and affects the respiration of dogs and rabbits in a characteristic manner. A pituitary extract that has been prepared in such a way as to be quite free from blood pressure-lowering and

broncho-constricting substances no longer affects the bronchi—unless it be in the way of causing a slight dilatation when given in great excess. Similarly the tartrate of the specific principle of the posterior lobe fails to cause the slightest constriction of the bronchi and in very large doses causes a slight dilatation.

We have so far failed to find a single activity of a posterior lobe extract referable to a specific principle or principles which is not duplicated by our tartrate and we therefore conclude that the oxytocic, pressor, respiratory, diuretic-antidiuretic actions of our tartrate are but manifold actions of one and the same active principle or hormone.

Further evidence in favor of this belief is seen in the following facts: (a) All of the physiological properties of our active substance increase in intensity and in the same ratio *pari passu* with the methods of purification, from the initial precipitation of the fresh posterior lobe substance to the final precipitation of the tartrate. It is unlikely that all of these physiological properties would retain their relative position to each other throughout a long series of varied chemical processes were they not characteristic of one and the same principle. (b) Exposure of the tartrate to normal sodium hydroxide for one hour at room temperature *completely destroys all* of its physiological actions. It is extremely unlikely that so comparatively mild a treatment as this should simultaneously destroy three or four separate principles. (c) Boiling a solution of the tartrate with 1 per cent. hydrochloric acid under a reflux condenser for one-half hour is also destructive of all these physiological properties, with the exception of the oxytocic action, a certain proportion of which appears to withstand this short treatment with acid.

A communication has just been received from the pharmacological laboratory of Professor G. Modrakowski of Warsaw which supports my contention that the posterior lobe contains only one specific hormone. Doctors Leyko and Sikorski of that Institute have studied the blood pressure-raising power of post-pituitary extracts on dogs at intervals of two days, the operation being performed under local anesthesia. The action of the extracts

upon the urinary secretion of dogs with a permanent bladder fistula was then studied and it was found, as in their previous experiments (1921), that the extracts promptly check the diuresis induced by the copious administration of water to the animals. The pressor and antidiuretic action of the extracts were then compared with their oxytocic action on the guinea pig's uterus and it was found "that these three actions go together." To cite a passage from the concluding section of their paper: "An extract giving a strong pressor effect acts with about equal strength upon the diuresis and *vice versa*. This seems to speak in favor of Abel's view that the extract contains only one main active principle acting in all three directions, and against the presence of a number of different active bodies in the gland, each acting only in one way."

Smith and McClosky in a paper soon to be published in the *Journal of Pharmacology and Experimental Therapeutics* and from which I have the privilege of quoting have also made a valuable contribution to the point under discussion. These investigators "have studied the diffusion rate of the several physiologically active constituents of the infundibulum through a series of collodion membranes of graded permeability," and conclude that "the identical diffusion rate of the oxytocic, pressor and renal activities present in infundibular extracts argues in favor of their chemical identity, and confirms the view held by Abel and his collaborators on this matter."

But while the posterior lobe and pars intermedia contain but one specific principle possessing these manifold physiological properties, it must be borne in mind that it also contains in small amounts two depressor substances which cannot be said to be characteristic of this part of the hypophysis, although both are found in larger amount in the posterior lobe than in the anterior lobe. These two blood pressure-lowering substances have been named the "B" and "C" substances in an earlier paper (Abel and Nagayama) in which also the specific principle of the posterior lobe which has now been separated in the form of a highly active tartrate was named "A". The two depressor

substances, " B " and " C ", are completely separated from the pressor-oxytocic-diuretic-antidiuretic substance " A " by our chemical methods as will be shown presently. " C " is histamine. This base was first isolated in the form of a pure picrate from the dried whole pituitary by Abel and Kubota in 1919. At that time I supposed that this base represented the entire oxytocic value of the posterior lobe, taking for granted in those days that this lobe contains several active principles. My own further studies in this field, as also the communications of Jackson and Mills and of Dudley showed me that I had exaggerated the rôle of histamine in pituitary extracts. I at once admitted the correctness of the position assumed by these writers, that the real uterine stimulant, the " A " substance above referred to, and histamine are two distinct chemical individuals. It was then intimated by some writers (Hanke and Koessler) that the histamine which Kubota and I had isolated from the hypophysis was probably of bacterial origin. This suggestion has been proved by Roca in my laboratory to be entirely unfounded. Roca has demonstrated that histamine or the " C " substance is obtainable by mild treatment with acids from the hypophysis when the glands are gathered at the slaughter house and are either immediately placed in boiling water or sliced into corrosive sublimate solution as they are taken from the skulls of the beeves. Histamine is not present in the free form or as a simple salt in the entirely fresh gland, but it always appears when the ground-up hypophysis is boiled with acids in the usual process of deproteinization of the extracts. It is also present, in a small amount to be sure, in our commercial extracts as has been shown by Jackson and Mills and in my own laboratory. This agent, together with the " B " substance, is responsible for the broncho-constrictor and perhaps other actions which are not given by the purified hormone, or " A " substance. All of the earlier commercial extracts, which were often boiled for too long a time and in the presence of too much acid, always contained more depressor material than do the commercial extracts of the present day. Roca has shown that, after destruction of the " A "

substance by acid, a posterior lobe extract will be 7 to 8 times more depressant for the arterial pressure than is a similarly treated extract of the anterior lobe and that chloroform takes up from the posterior lobe material thus treated about 20 times more histamine than is obtainable from the anterior lobe similarly treated. Abel and Kubota have shown that the precursor of histamine, from which histamine is easily liberated by acid hydrolysis and which cannot be histamine, is widely distributed in the body, being present in the liver, the gastric mucosa and elsewhere.

A second depressor substance, " B ", has been described by Abel and Nagayama. It differs from histamine in being entirely insoluble in chloroform and also in other respects. It has not as yet been prepared in any other form than as an amorphous water-soluble residue, soluble in 90 per cent. alcohol and which has the properties of an albumose. It gives the biuret reaction and can be salted out with ammonium sulphate or with sodium chloride. It corresponds in its physical and physiological properties with a similar chloroform-insoluble depressant material that can be separated from tissue extracts in general. It is therefore a widely distributed principle and it is to be regretted that no extensive attempts towards its purification and isolation have been made.

To recapitulate my position: Extracts of the pars intermedia and the posterior lobe of the bovine hypophysis contain only one specific hormone, the " A " substance of Abel and Nagayama, which has been isolated in the form of a more or less pure tartrate and which exhibits all of the manifold characteristic physiological actions of the posterior lobe. My position is diametrically opposite to that of certain other investigators (Fühner, Dale and Dudley) who assume that at least three and even four or more (Fühner) specific hormone principles exist in the extracts of the posterior lobe.

In a properly made extract of the posterior lobe this powerful " A " substance entirely overbalances the action of the two other substances which are always present to a small extent, the amount

being greater when an extract has been exposed to the action of weak acid and heat for too long a time. The earlier literature on this subject contains numerous blood pressure tracings whose character shows at a glance that the authors in question were working with damaged extracts. It is seen in such tracings that the pressor effect does not immediately follow upon the injection of the pituitary extract as should be the case, but is preceded by a fall in the blood pressure which is often very pronounced and it is only after this depressor action has worn off that the belated pressor effect is observed. It is only such damaged pituitary extracts that induce in the dog a pronounced broncho-constrictor action.

One of the two minor substances, " C ", is histamine which exists in the hypophysis as in many other locations in the body in the form of a labile precursor of unknown properties and composition. The other or " B " substance, also a blood pressure-lowering and mildly oxytocic agent, differs from histamine in being entirely insoluble in chloroform, giving the biuret reaction and having the properties of a proteose, as at present isolated. I would, however, not be understood as saying that the substance is in reality a proteose. It may be a substance of simple composition which is merely admixed with or adsorbed on proteoses.

OUTLINE OF METHOD OF ISOLATION

Preparation of the mercuric chloride-protein cake. The whole bovine glands are placed in cold storage at the slaughter house immediately after their removal from the skulls of the animals: the following morning the posterior lobes are trimmed out and ground to a very fine paste and each 100 grams of this paste is thoroughly mixed with 100 c.c. of 0.35 per cent. hydrochloric acid containing 4 grams of mercuric chloride and placed in a tightly stoppered bottle of such a size that the air space above the contents is as small as possible. When the mixture is received at the laboratory, it is treated with 10 grams of solid mercuric chloride, shaken several hours and filtered by suction. The insoluble " cake " is washed twice by grinding with about

25 c.c. of saturated mercuric chloride solution and filtered by suction. During the past winter we have found it advisable to depart somewhat from the above procedure. The glands are chilled as before, but the separated posterior lobes (plus pars intermedia) are defatted with acetone after being first ground to a fine paste. The defatted material reaches us in the form of a white powder which is ground up with solid mercuric chloride and 0.2 per cent. hydrochloric acid solution. The mixture is then shaken for several hours in a shaking machine and the cake is washed twice by grinding with saturated mercuric chloride solution and filtering by suction.

The pars intermedia of the hypophysis of the ox closely invests the posterior or neural lobe and cannot readily be separated from it. In our work it was naturally always utilized along with the neural lobe. Unless otherwise stated all so-called bovine posterior lobe extracts are in reality extracts of these two divisions of the hypophysis, both of which contain the active principle under discussion. That the pars intermedia substance can be obtained in the pure condition and that extracts made from it have a pressor and other physiological actions has been amply proved by Herring, Lewis, Miller and Mathews, Biedl, Houssay, Atwell and Marinus and others. Osborne and Vincent concluded from their experiments that an extract made from the central part of the posterior lobe—devoid of epithelial elements—is much more active in raising the blood pressure than one made from the peripheral epithelial part and expressed the opinion that probably the external layer would be found to be inactive if it could be obtained quite free from admixture with the central portion. Schafer and Herring also found the central portion of the neuro-hypophysis to be more active as a diuretic than the external layer. In Vincent's exhaustive review on the functions of the hypophysis (1915) the work of Foderà and Pittau, of Salvioli and Carraro, and of Houssay and his pupils is cited in support of his conclusion "that it is the nervous portion proper of the organ which contains the substance or substances having such pronounced pharmacodynamical properties

different from those possessed by other kinds of nervous tissues" and he further finds himself "strongly inclined to adopt the view of Houssay that the substance or substances are secreted by the cells of the pars intermedia, and then collected and concentrated or changed into more active forms in the nervous portion proper."

The investigations of Atwell and Marinus (1918) harmonize many of the differences that are found in the observations of their predecessors. These writers have also shown that the weak physiological effects given by extracts of the pars tuberalis are due to the inclusion of some of the neural stalk substance during the preparation of the extract. In other words pure extracts of the pars tuberalis are devoid of the pressor, oxytocic and other actions so characteristic of the posterior lobe extracts. They were also able to prepare pure extracts of the neural portion of the hypophyseal stalk which were found to have an action on the blood pressure of the dog and on the guinea pig's uterus in every way comparable to those obtained from a commercial pituitary extract. It may be accepted as proved that properly made extracts of the pars intermedia, of the neural portion of the hypophyseal stalk and of the posterior lobe itself are practically all of equal potency and exhibit all of the characteristic reactions of the posterior lobe extract.

An explanation of the contradictory findings of physiologists, not only in respect to the relative strength of the extracts of the several divisions of the hypophysis, but also in respect to the location of the several postulated active principles, is now found in our fuller knowledge of the chemical properties of the real active principle. The ease, for example, with which this sensitive hormone can be altered in its properties by boiling at the wrong pH was unknown to our predecessors and this circumstance is probably more accountable than any other for the confusion which has hitherto characterized the physiological as well as much of the chemical literature of the subject.

Liberation of the active principle from the "cake" and precipitation with phosphotungstic acid. The washed cake is

ground with about 200 c.c. water and the resulting suspension is neutralized to litmus paper with sodium hydroxide, treated with hydrogen sulphide in excess, then with about 40 c.c. of saturated sodium chloride solution (to flocculate the precipitate of mercuric sulphide) and filtered with the aid of very gentle suction. The filtration is quite slow and if too much suction is applied the paper easily becomes entirely clogged. The filtrate is aerated to remove the excess of hydrogen sulphide, treated with a 1:1 solution of phosphotungstic acid until precipitation is complete and filtered by suction.

Decomposition of the phosphotungstate precipitate and precipitation with tannic acid. The precipitate is ground with ice-cold water and enough sodium carbonate solution to make the turbid solution barely alkaline to litmus paper and reprecipitated with hydrochloric acid, the solution in sodium carbonate and reprecipitation with hydrochloric acid being repeated twice more. Generally the first treatment with sodium carbonate leaves undissolved a small amount of yellow residue which is usually filtered off, but no effort is made to obtain a clear filtrate. The reprecipitated phosphotungstate is finally taken up in dilute sodium carbonate solution and treated, ice-cold, with hot saturated barium hydroxide solution, drop by drop, until precipitation is complete and filtered from the barium phosphotungstate. To the filtrate dilute sulphuric acid is added until no further precipitate is formed; together with the barium sulphate there is thrown down a small amount of a flocculent substance which apparently carries down some of the hormone by adsorption, for when the mixed precipitate is treated with dilute sodium carbonate solution and filtered the filtrate always shows more or less oxytocic activity. The filtrate from the precipitate formed by the sulphuric acid is neutralized with sodium hydroxide and treated with a concentrated aqueous solution of tannic acid and then with solid powdered sodium chloride until the latter no longer dissolves, the mixture being thoroughly stirred throughout the addition of both the tannic acid and the salt. The precipitated tannate is filtered off and more tannic acid and salt are

added to the filtrate; this produces a second precipitate, the filtrate from which is treated a third time with tannic acid and salt. The final filtrate is now usually found to be entirely devoid of any action on the uterus. The combined tannate precipitates are washed by grinding several times with saturated sodium chloride solution and filtering by suction, and are pressed as dry as possible on the funnel with a glass rod.

Conversion into the tartrate. The tannate precipitate, while still moist, is ground with a little 95 per cent. alcohol and 1 or 2 c.c. of a saturated alcoholic solution of tartaric acid and the resulting thick solution is pipetted off from the undissolved residue, which is again ground with a little alcoholic tartaric acid, the process being repeated until the residue, which consists largely of sodium chloride, is practically colorless. The alcoholic tartaric acid extracts are treated with much anhydrous ether (at least 20 volumes) and the resulting precipitate is again taken up in alcoholic tartaric acid and reprecipitated with ether, the process being repeated three times.

The tartrate is now collected on a hardened paper and immediately transferred to a vacuum desiccator charged with phosphorus pentoxide. It has been our experience during the past few years that our salts remain unaltered when dried in this way while the tension of sulphur trioxide in desiccators charged with sulphuric acid appears to be great enough to injure our labile substance. This seems also to have been the experience of Burn and Dale.

Purification with picrolonic acid. The tartrate is ground with a little water and, without removing the small amount of material which may remain undissolved, is treated, ice-cold, with a saturated solution of picrolonic acid in alcohol. The considerable precipitate thus obtained consists of foreign picrolonates and carries down with it only a small amount of the active material. The precipitate is removed by filtration and more of the alcoholic solution of picrolonic acid is added together with some cold water. An additional amount of the foreign inactive picrolonate is thus

removed. This process is repeated as long as any considerable amount of picrolonates can be precipitated.

We have here a good instance of non-adsorption as compared with the earlier precipitates, which adsorb the hormone quantitatively. The clear supernatant fluid containing the active principle is shaken a few times with ether to remove the excess of picrolonic acid and is then poured into small shallow glass bowls in which it is allowed to evaporate in a current of air at room temperature. When the solution has become rather concentrated it is again tested with picrolonic acid in alcohol and if it again gives a considerable precipitate, the process of purification outlined above is repeated and the solution is allowed to evaporate to dryness. The air-dry residue is taken up in 93 per cent. alcohol (which may profitably contain a small amount of tartaric acid) and precipitated with much ether. The precipitate is washed with alcohol-ether (1:20) and dried over phosphorus pentoxide. The tartrate thus obtained is highly active for the guinea pig's uterus. According to the care with which the above operations have been carried out and more especially when the injurious action of acids or other agents has been avoided, this tartrate will be found to be from 125 to 250 times more powerful in its action on the uterus than the acid phosphate of histamine.

Nature of the precipitate obtained with picrolonic acid. The precipitated picrolonates appear to consist mainly if not entirely of biuret-yielding proteoses. These are recovered from the precipitate by the simple process of dissolving them in acidulated water (HCl), removing the freed picrolonic acid by repeatedly shaking with ether and allowing the aqueous fluid to go to dryness in a current of air at room temperature. The residue gives no signs of crystallizing, gives the biuret reaction with great intensity and is of low oxytocic value (only $3 \times \beta$ -1 phosphate). By repeatedly salting it out from its solution in water by the addition of powdered sodium chloride, the oxytocic titer of this substance may be still further lowered. This proteose-like body also gives a Pauly reaction and when warmed gently with hydrochloric acid gives a pink to plum color (Guggenheim's

reaction). By the use of picrolonic acid a very considerable amount of this substance may be removed from the crude pituitary tartrate. Thus in a purification experiment recently made with this reagent, I started with 0.760 gram of a crude tartrate whose oxytocic titer was somewhat better than $50 \times \beta$ -phosphate. The picrolonates obtained from this weighed 0.2828 gram and the biuret-yielding substance recovered from this amount of picrolonates weighed 0.1170 gram, which, however, did not represent the entire amount obtainable as some of the material was used in making tests before it was weighed. When we take into account the small amount of active principle that adheres to the precipitated picrolonates and how large an amount of material is removable by this method, it will be seen that this step in our purification process is an advantageous one.

Further purification of the active tartrate. The further purification of the tartrate is effected by means of solvents and naturally, from this point on, is achieved only at the cost of the scattering of the active substance, since a sharp separation into entirely inactive and highly active fractions is impossible with the use of solvents alone. Nevertheless, it is possible, at this stage of the operations, to carry a tartrate with an activity of from 50 to 250 times that of histamine acid phosphate to a stage where it is 1000 to 1250 times as powerful as the histamine salt.

We hope to elaborate better methods for these final stages of the purification, that is to say, methods more nearly comparable to those involving the use of tannic or picrolonic acid rather than fractional precipitation with organic solvents. For that reason we give only a mere outline of our work with solvents as at present carried out by us.

Our active principle in the form of a "tartrate," if it may be described as such, as seems permissible from our procedure, is extremely soluble in 93 to 95 per cent. alcohol, in water and in pyridine, much less soluble in absolute ethyl alcohol and in dry *n*-butyl alcohol, fairly soluble in methyl alcohol and quite insoluble in dry ether, chloroform, acetone and benzene. A very

considerable increase in activity may be brought about by the expert use of absolute alcohol alone at room temperature. The judicious addition of *n*-butyl alcohol to a solution of the tartrate in 95 per cent. alcohol throws out a fraction of less oxytocic activity while a more highly active fraction remains dissolved in the mixed solvents and can be precipitated out with ether. The precipitate is then repeatedly extracted with absolute alcohol and the combined extracts are allowed to evaporate to a small volume in an air current at room temperature. This is the method which we have generally used to obtain our most highly active preparations. Pyridine also dissolves the tartrate, with its accompanying inactive impurities, with the greatest ease; from this solution a less active fraction can likewise be precipitated with *n*-butyl alcohol or absolute alcohol plus a trace of ether. In either case, after the filtrate from the relatively inactive precipitate has been concentrated to a small volume at room temperature the highly active tartrate is precipitated with a large volume of ether and the precipitate is washed with absolute alcohol-ether (1:20) and quickly placed in a phosphorus pentoxide desiccator, so that no moisture condenses on it as the ether evaporates. In these ways 0.01 to 0.03 gram or more of dry white substance varying in oxytocic value from 600 to 1250 times histamine acid phosphate is obtained at a time. It may be noted here that this tartrate is now no longer separable by *n*-butyl alcohol into fractions which differ in their physiological action. Although the yield of the purest tartrate is not large, as we have just seen, that of the impure or first tartrate is generally quite satisfactory, considering the difficulties that are here encountered. For example, as much as 0.305 gram of the first or impure tartrate, with an oxytocic value of 50 to 75 times β -I phosphate, was recently obtained from 225 grams of fresh infundibular substance. In this instance no special measures were taken to insure a high oxytocic titer of this first product. Naturally, when the greatest precautions are taken and one or other step in the process is repeated, the first tartrate will generally

have a much higher oxytocic value, but the yield will be less in quantity.

NATURE OF THE FINAL PRODUCT

We cannot yet claim that our final product, with its very high activity for plain muscle tissue and for the vascular and the renal apparatus, represents a single chemical individual. The repeated use of various solvents, as just outlined, appears, however, to have removed every trace of readily crystallizable material. When, for example, the tartrate which, we will say, has an oxytocic value of 250 times histamine acid phosphate, is dissolved in 93 per cent. alcohol and the solution is allowed to evaporate a considerable amount of substance separates out in the form of beautiful, colorless, branching, fern-like crystals which give a positive Pauly but no biuret reaction. This material is devoid of physiological activity, at least as far as its action on the uterus, blood pressure and the kidney are concerned. The highly active remainder, on the other hand, when dissolved in 93 per cent. alcohol or water and then allowed to evaporate, dries down to a colorless or nearly colorless amorphous layer.

The addition of 10 per cent. sodium hydroxide solution to the tartrate (even the purest specimen) instantly liberates a gas which is alkaline to litmus and which smells strongly of an alkylamine mixed with ammonia. On adding a few drops of concentrated hydrochloric acid to a minute amount of the dry tartrate and warming gently over the free flame, a beautiful pink or violet color immediately appears. On standing this color gradually changes to brown. We cannot be sure at this writing that the tartrate of highest purity will still give this test as we have not applied it to preparations with an oxytocic value of more than 160 times β -I phosphate. This color reaction was first observed by Guggenheim when working with the pituitary extract known as "Pituglandol."

Neither picric nor picrolonic acid precipitates anything from an alcohol or aqueous solution of our final tartrate. Mercuric chloride and tannic acid (without sodium chloride) also fail to yield a precipitate. Phosphotungstic acid precipitates the active

principle only very incompletely, even when the tartrate has only the value of 250 times histamine acid phosphate, a fact which leads us to suspect that this reagent will no longer precipitate the fully purified substance. The Pauly reaction is still given by our most highly active preparations, but as regards the biuret reaction our impression, at the moment of writing, is that it is not given with the same intensity as by less highly purified preparations and we must therefore reckon with the possibility that an albumose- or peptide-like substance still contaminates our product unless indeed the active principle is itself a peptide-like substance.

In spite, therefore, of the extraordinary potency of our final product, we cannot be certain that we are dealing with a single chemical individual; on the contrary, we suspect that it still contains an admixture of some inactive constituent or constituents which are most likely proteose-like substances.

COMMENTS ON THE FOREGOING METHOD OF ISOLATION

It is evident that the pars intermedia and the pars posterior of the pituitary gland cannot contain more than a very small amount of our active principle. A gram of fresh pars intermedia plus pars posterior substance contains on an average an amount of oxytocic principle equivalent to 0.25 gram of histamine acid phosphate and since our purest specimen of the actual pituitary "hormone" is at least 1250 times more powerful than the histamine salt, it follows that one gram of infundibular tissue cannot contain more than 0.0002 gram (0.02 per cent.) of this singular and potent substance. And it becomes evident at once that one cannot hope to isolate a principle present in such minute amounts from so complex a tissue as the pituitary infundibulum unless one can use methods by which it is possible to carry the principle along from step to step without material loss. The methods described above, when properly used, meet this requirement. They are based on the fact that the active principle is adsorbed by various flocculent precipitates which offer an enormous surface. But this procedure would have defeated our pur-

pose had we not been able to discover methods for liberating the principle from the surface on which it is adsorbed.

The points involved in the use of mercuric chloride as a precipitating agent furnish illustrations of the principles here involved. When the finely minced infundibular substance is mixed intimately with an excess of acidulated mercuric chloride solution the active principle is quantitatively adsorbed by the mercuric chloride-protein "cake." After disintegrating the cake and precipitating the mercury with hydrogen sulphide, the active principle can be liberated only by adding a sufficient excess of sodium chloride, which causes the colloidal mercuric sulphide to coagulate and so alters the surface conditions that the hormone is no longer adsorbed. In this connection it may be pointed out how greatly the various constituents of the neurohypophysis differ in their adsorbability by the mercuric chloride-protein cake, a difference which makes it possible to effect a clean-cut separation of substances that act on the blood pressure. From the cake itself we obtain only the pressor-oxytocic principle while the filtrate from the cake yields all of the preformed depressor substances present in the ground and acidulated infundibulum. Now mercuric chloride will not *reprecipitate* the principle from the solution obtained from the mercuric chloride-protein cake by the use of hydrogen sulphide and sodium chloride. Evidently, the large adsorbing surface of the precipitated proteins are necessary in order to bring down the hormone. However, a very simple expedient makes it possible to use a mercury salt as a precipitant a second time or as often as may be desired. It is only necessary to add first a sufficient quantity of disodium hydrogen phosphate to the solution of the hormone and then to add a solution of mercuric nitrate. The flocculent precipitate of mercuric phosphate evidently offers a suitable surface since, under the above conditions, the hormone is precipitated quantitatively. Here also, the free use of sodium chloride is necessary, in the precipitation of the mercury with hydrogen sulphide, in order to prevent adsorption. We have recovered as much as 90

per cent. of a known quantity of our principle by this method of reprecipitation with mercuric nitrate.

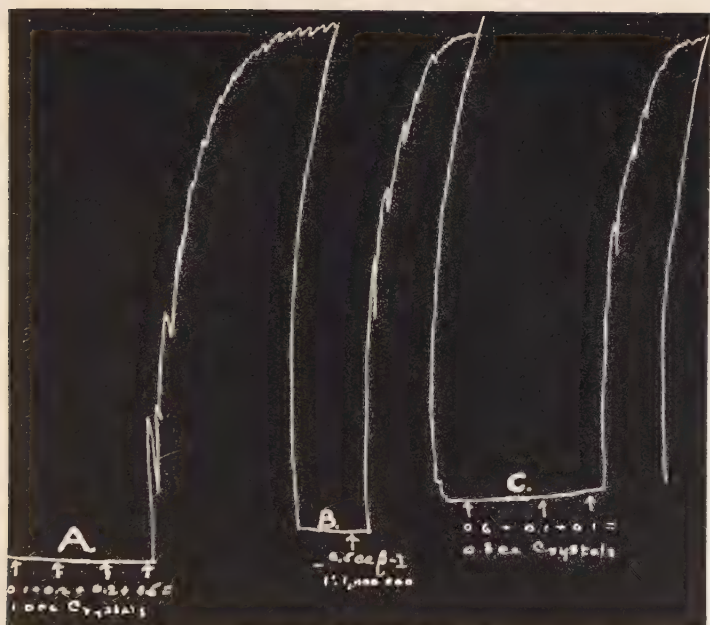
Phosphotungstic acid also precipitates the principle quantitatively at the stage at which we employ this reagent. Later in our work, as concomitant inert substances were progressively removed it was found that the phosphotungstic acid no longer precipitated the principle quantitatively and we therefore suspect that here, as in the case of the mercurials, the hormone is only adsorbed on the surface of (other) precipitated substances.

Tannic acid also acts in this way, *i.e.*, it serves to furnish the necessary surfaces for adsorption as it falls out in flocculent form in the presence of sodium chloride. This acid yields no precipitate whatever when added to a solution of our purified tartrate which is free from electrolytes. The ready solubility of the precipitated tannates and of the excess of tannic acid in an alcoholic solution of tartaric acid and the relative insolubility of the tartrate of the hormone in an alcohol-ether medium makes it possible to remove the active principle from the tannic acid precipitate and obtain it in the form of a dry tartrate.

On the other hand, in picrolonic acid we have an agent which removes impurities from an aqueous solution of the hormone with only a trifling loss of the latter by adsorption. In this case the precipitated foreign picrolonates soon aggregate to yellow resinous lumps with no extensive adsorbing surface.

We hope that our methods of allowing our principle to attach itself to adsorbing surfaces from which it may again be liberated without serious loss will be capable of further improvement.

There is, of course, nothing novel in the ideas underlying the above methods. Other investigators in this and in other fields of chemical work have repeatedly noted that this or that active principle is often lost by what were thought to be instances of irreversible adsorption. It will be seen, however, that we have been fortunate enough to be able to turn to our advantage some of the instances of supposedly irreversible adsorption and to develop them into useful methods of isolation.



CRYSTALLINE PRODUCT 50 TO 62.5 TIMES STRONGER THAN HISTAMINE
ACID PHOSPHATE.

Fig. 1.—Contraction of the virgin guinea-pig's uterus, suspended in 30 cc. Tyrode solution, produced by a 1:1,000,000 solution of histamine acid phosphate and by a 1:100,000,000 solution of a product consisting of a lump of well formed prisms which crystallized out of a concentrated solution of a pituitary tartrate in pyridine. After one washing with pyridine and then with alcohol-ether (1:20), the lump weighed 4.7 mgm. This product was not recrystallized, as previous experience had taught us that products of this sort did not retain their activity on recrystallization. A. 1.0 cc. of crystalline product (0.00001 mgm. or 1:3,000,000,000). B. 0.5 cc. of histamine acid phosphate (0.0005 mgm. or 1:60,000,000). C. 0.8 cc. of crystalline product (0.000008 mgm. or 1:3,750,000,000).

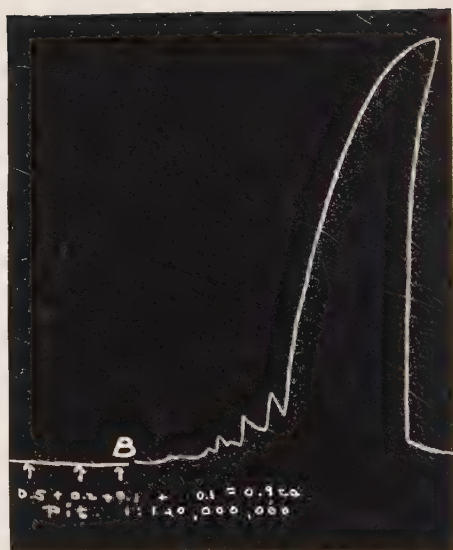
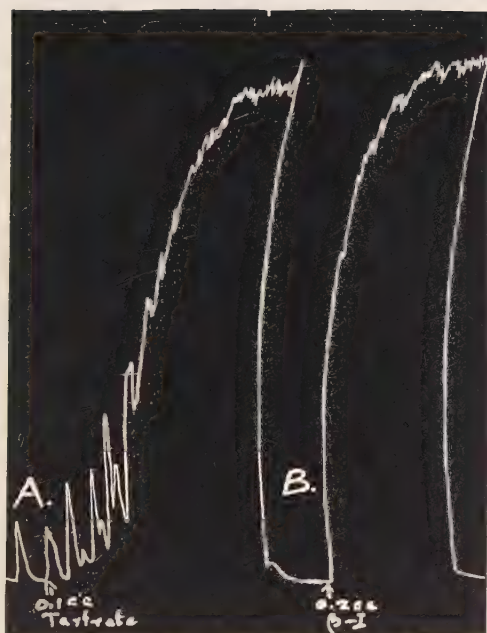


FIG. 2.—Pituitary tartrate 550 times stronger than histamine acid phosphate. Contraction of the virgin guinea-pig's uterus, suspended in 30 cc. Tyrode solution, produced by a 1:100,000 solution of histamine acid phosphate and a 1:100,000,000 solution of a pituitary tartrate. A. 0.5 cc. histamine acid phosphate (0.005 mgm. or 1:6,000,000). B. 0.9 cc. pituitary tartrate (0.000009 mgm. or 1:3,300,000,000).



PITUITARY TARTRATE 1000 TIMES STRONGER THAN HISTAMINE ACID PHOSPHATE.

FIG. 3.—Contraction of the virgin guinea-pig's uterus, suspended in 30 cc. Tyrode solution, produced by a 1:50,000,000 solution of a pituitary tartrate and a 1:100,000 solution of histamine acid phosphate. A. 0.1 cc. pituitary tartrate (0.00002 mgm. or 1:15,000,000,000). B. 0.2 cc. histamine acid phosphate (0.002 mgm. or 1:15,000,000).



FIG. 4.—Pituitary tartrate 1250 times stronger than histamine acid phosphate. Contraction of the virgin guinea-pig's uterus, suspended in 30 cc. Tyrode solution, produced by a 1:250,000,000 solution of a pituitary tartrate and a 1:100,000 solution of histamine acid phosphate. A. 0.4 cc. pituitary tartrate (0.0000016 mgm. or 1:18,750,000,000). B. 0.2 cc. histamine acid phosphate (0.002 mgm. or 1:15,000,000).

PHYSIOLOGICAL PROPERTIES OF THE PITUITARY TARTRATE

The following tracings give evidence of the various physiological actions—pressor, oxytocic and diuretic—of our substance. Figure 1 illustrates the action on the uterus of a product which, although crystalline, contained so much of the active principle admixed or adsorbed that its oxytocic value was 50 to 62.5 times that of histamine acid phosphate; figure 2 is the tracing obtained with an amorphous tartrate which was 550 times stronger than the histamine salt, and figures 3 and 4 show that our most powerful tartrates varied in their oxytocic value from 1000 to 1250 times histamine acid phosphate.

It is interesting to note that our tartrate not only induces uterine contractions at these high dilutions of 1:18,750,000 or more (depending upon the sensitivity of the uterus) but also raises the arterial pressure to an appreciable degree in doses of 0.01 mgm. in cats, causes a marked diuresis in rabbits in doses of 0.05 mgm. or less, and also affects in a characteristic manner the respiration both of the dog and the rabbit, more especially in the unanesthetized animal.

In proof of this last statement we cite from a protocol of an experiment made on April 13, 1923, in which 0.2 mgm. of our tartrate, with an oxytocic titer of 250 times β -I phosphate, when injected intravenously into an unanesthetized dog weighing 13 kilos, produced the typical effects noted after the administration of the commercial pituitary preparations. Immediately following the introduction of the tartrate a very marked blanching of the skin and visible mucous membranes becomes apparent. A few minutes later the animal begins to have panting respirations at a rate of 60 or more per minute. These are of brief duration and periodic in character, with apnoeic intervals—Cheyne-Stokes in type. This respiratory effect of our tartrate on unanesthetized dogs has lately been verified several times. It is in every respect similar to the respiratory effect which was first shown to be characteristic of the action of hypophyseal extracts by Fühner and Pankow. These writers very aptly describe the respiratory tracings obtained by them in their experiments in urethane-

anesthetized rabbits as " Spindel-form " curves. This striking action on the respiration of unanesthetized dogs does not follow the injection immediately, but only several minutes later, a fact which makes it appear that the effect is of a secondary character, coincident with the very decided and general capillary anemia and dryness of the mouth and mucous membranes which are such striking and immediate results of the intravenous injections of our tartrate and of the commercial pituitary extracts. The experiment just described had for its purpose the study of the effects of our principle on the blood pressure of the unanesthetized dog with Kolls' apparatus. We would remark in this connection that we are dealing with a principle whose action on the circulatory and respiratory apparatus is much more marked in unanesthetized than in anesthetized animals as has been observed in this laboratory by Kolls and Geiling.

Figure 5 illustrates the action of our tartrate in urethane-anesthetized rabbits. It is seen that our active principle induces the same respiratory changes (" Spindel-form " curves) that were obtained by Fühner and Pankow with pituitary extracts and later with the " Hypophysin " of the Hoechst chemists.

In reference to the action of our tartrate on the arterial pressure, I would here point out as I have also done in a series of papers which have come from my laboratory that this salt is quite free from all preformed depressor substances which are usually mixed with it in the ordinary commercial extracts. As has been stated in the description of the chemical processes, these accompanying depressant substances are completely separated during the early stages of those processes. As a consequence of the absence of depressant substances the arterial tracing, following an intravenous injection of the tartrate, generally mounts straight upward and does not show that pronounced downward dip which is so characteristic of the tracings obtained with pituitary extracts which have suffered injury in the course of preparation. Even with tartrates of the lower oxytocic titer of say 40 to 75 times β -I phosphate we only rarely find a small downward dip preceding the steep rise in pressure and it is prob-



EFFECT OF PITUITARY TARTRATE (50 I PHOSPHATE) ON THE RESPIRATION AND BLOOD PRESSURE OF A
URETHANE-ANESTHETIZED RABBIT.

Fig. 5.—Rabbit, weight 2 kg. Urethane, 1.5 grams in 15 cc. sub-cut. "A." Injection of 1 cc. (0.3 mg.) of pituitary tartrate. Upper tracing, respiration; middle tracing, blood pressure; lower tracing, time in seconds.

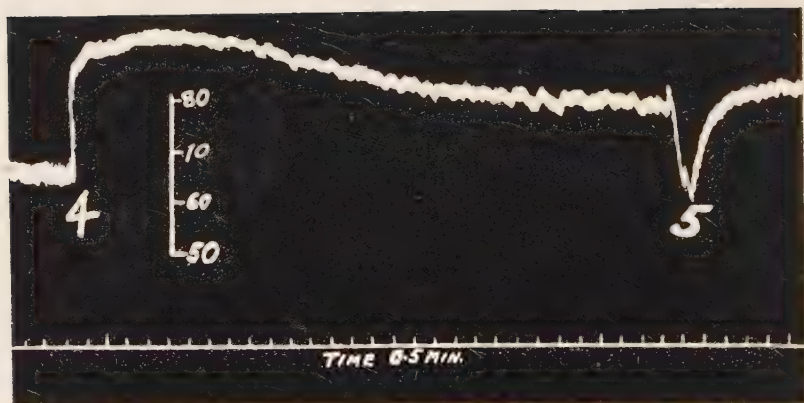


FIG. 6.—Cat, female, weight 2.8 kgm. Ether anesthesia. Carotid blood pressure. Pressor and inversion effect of an impure tartrate fraction which was thrown out of *n*-butyl alcohol by dropping into it a solution of the tartrate in 93 per cent. ethyl alcohol. The oxytocic value of this fraction was 40 to 50 times β -I phosphate. At (4) 0.5 cc. (= 2.95 mgm.) was injected intravenously. This injection, however, was preceded at intervals by three injections of 1 cc. each of Armour's pituitary extract, diluted 1:100 without causing an inversion. The pressor effect, therefore, of the tartrate was much smaller than would ordinarily be the case. At (5) a second and much larger dose, 2 cc. = 8.75 mgm., of the tartrate was injected intravenously and this induced an inversion effect on the blood pressure as shown in the figure. This tracing is quite similar to many that Abel and Nagayama, and Abel and Rouiller obtained with preparations which were made in an entirely different manner, e.g., a phosphate and a tetranitroaniline compound. When the above tracing is compared with figures 6 and 7 it will be seen that this inversion effect is just as characteristic of the highly purified tartrates as of the cruder preparation used above. In a word, this property is retained by the purified salt which may exhibit it when injected in doses so small as 0.01 mgm.

able that this finds its cause in too rapid an injection of the solution.

A curious fact to which I have also called attention repeatedly in my papers on this subject is the complete inversion of the arterial tracing which so frequently follows upon later injections of the tartrate. I have named this effect the inversion effect. It is not invariably obtained; the same solution may give the effect in one animal and not in the next. It appears to depend upon a number of indeterminate factors such as the depth of anesthesia, the relative size of the first and subsequent doses, the number of and time interval between successive injections, and possibly the age of the animal. I cannot believe that this inversion effect can be explained by assuming that the tartrate is a mixture of two substances, one possessing a pressor and the other a depressor action, and that the action of the latter only becomes manifest when the vascular apparatus can no longer respond to the former. Against this view the following facts may be adduced. The purified pressor substance, as I have frequently pointed out, is easily injured by various chemical procedures so that its characteristic action is lost and a depressor effect appears in its place. Now in cases where the injury is only partial, that is to say, long before all the pressor substance has been destroyed, a preliminary depressor effect is obtained—an effect which is apparently proportional to the amount of the pure pressor substance which has been inverted. So also if a very small amount only of histamine (something above an inoperative minimum) be added to our pure tartrate its presence is always detectable by the fact that the arterial tracing shows, when this mixture is injected, a definite downward dip before the rise. There are other points in connection with this phenomenon which need not be taken up here which have been discussed in earlier papers. I shall give here only one tracing (Figure 6) which illustrates the character of the pressor action and also the inversion effect produced by a tartrate with an oxytocic titer of only 40 to 50 times β -I phosphate. Examples of the same sort of tracings, however, with a tartrate of a very much higher degree of purity

can be found in my papers. We may add here that it may happen that the same tartrate gives a good inversion effect on one animal and does not give it on a second animal for reasons unknown to us at present.

Diuretic action. Schafer and his co-workers, Magnus and Herring, discovered the diuretic action of extracts of the pituitary gland and localized this action in the pars intermedia and posterior part of the gland. There has been much controversy in regard to this property of pituitary extracts, and while I cannot here review the work of the various authors on this subject, I would however state that on the basis of our own investigations I must hold that our tartrate (and properly prepared extracts of the posterior lobe in general) has an undoubted diuretic action in the green-fed, properly prepared, urethane-anesthetized rabbit. My collaborators and I also hold that it is this diuretic substance itself which acts as a powerful *anti-diuretic* for rabbits under the proper experimental conditions and that it is this substance likewise which acts as the *antidiuretic* principle in diabetes insipidus. Even in the green-fed rabbits, as our experiments show, the diuresis is preceded by a temporary cessation of urinary flow. Figures 7, 8 and 9 show that our tartrate when intravenously injected into properly prepared rabbits is a rapidly acting and powerful diuretic but one of short duration only. When the effect of a particular dose disappears and the kidney has returned to its normal state of secretion, a second injection induces diuresis as before and it would appear that this effect can be obtained as often as may be desired. The kidney, in contradistinction to the vascular apparatus as a whole, does not become tolerant to repeated injections of the drug. I desire in this connection to call attention to a striking fact which is brought out in Figures 8 and 9. In Figure 8 it is shown that there is an entire absence of diuresis after the injection of a solution of the tartrate which has been boiled for one-half hour under a reflux condenser with 0.5 per cent. hydrochloric acid. So too, in Figure 9 it is shown that the diuretic action of the tartrate was practically obliterated after exposure of its solution

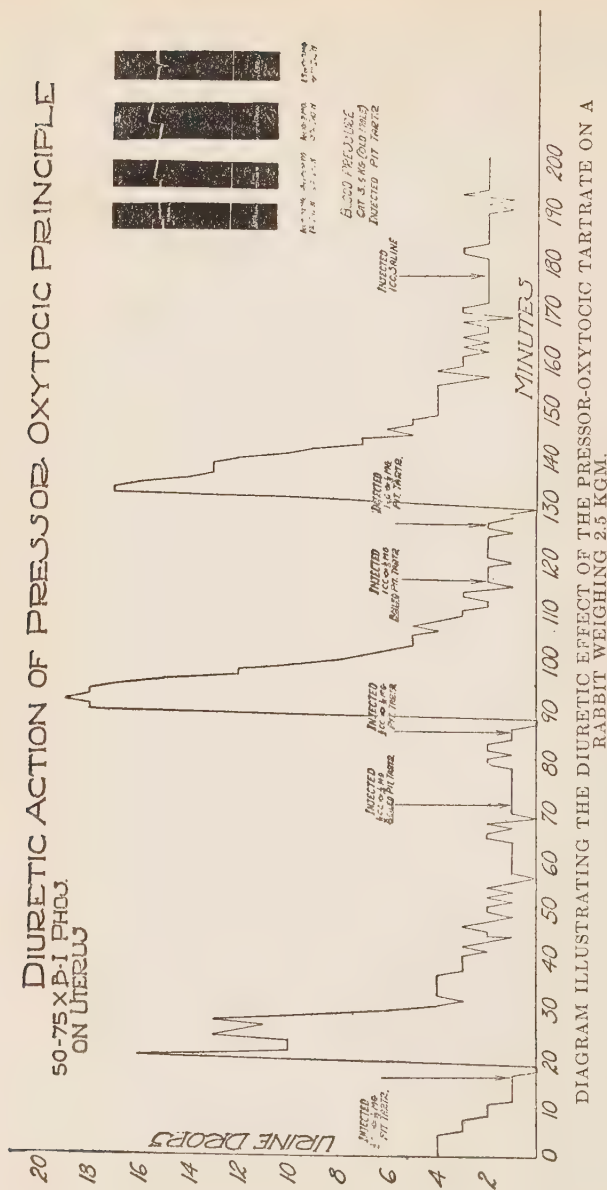


FIG. 8.—The animal had been fed with greens for a week previous to the experiments. Urethane introduced by stomach tube was employed as the anesthetic. The urethra was tied off and the bladder was cannulated with a small glass funnel firmly tied in place and practically replacing the organ, but allowing free communication with both ureters. The oxytocic value of this tartrate varied from 50 to 75 times β -I phosphate. The upper right hand corner of the chart contains four small tracings showing the pressor effect of the tartrate and the inversion effect on the fourth injection. In figure 7 these four blood pressure tracings are reproduced on a larger scale. From the above diagram it will be seen that there is a cessation of urine flow which lasts for about three minutes immediately following the injection of the pituitary tartrate. Thereafter a very striking diuresis occurs, the urine now being pale in color. Note the entire absence of diuresis following the injection of a solution of the tartrate which has been boiled for one-half hour under a reflux condenser with 0.5 per cent HCl.

EFFECT OF ALKALI ON THE DIURETIC ACTION OF THE PITUITARY PRINCIPLE

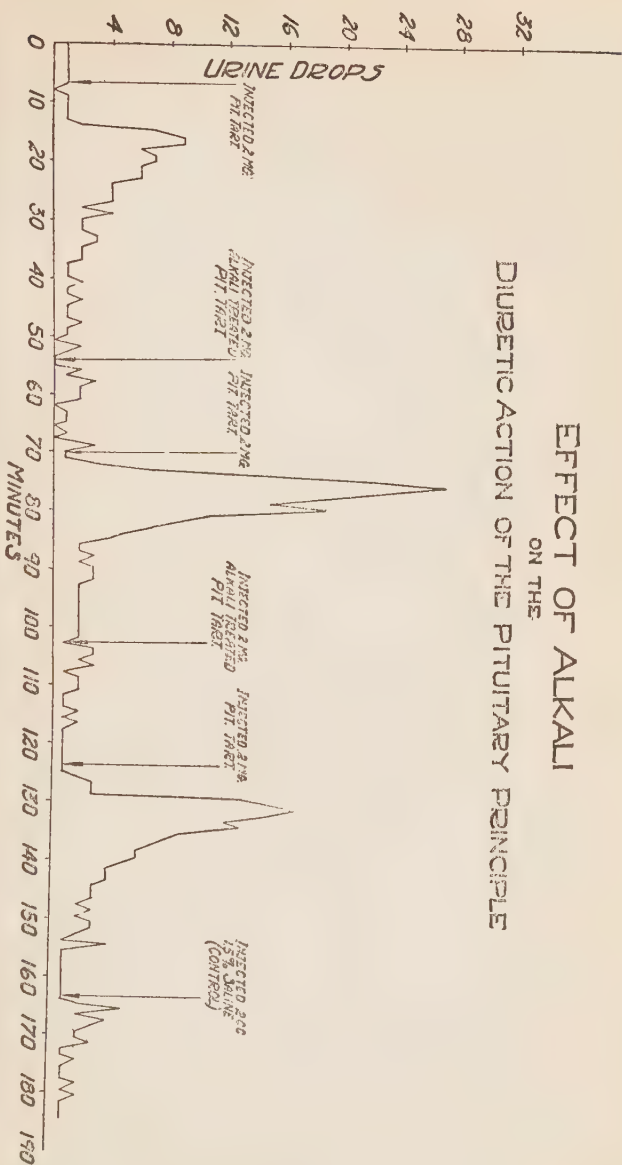


FIG. 9.—A rabbit weighing 2.8 kgm. and which had been fed greens for one week previous was used as the experimental animal. Anesthesia was induced by urethane given subcutaneously. The urethra was tied off and the bladder cannulated with a small glass funnel firmly tied in place and practically replacing the organ, but allowing full communication with both ureters. The pituitary tartrate used for this experiment is the same as that employed in experiments illustrated in figure 9. It will be noted from the chart that the diuretic action of the pituitary tartrate was practically obliterated after treatment with normal sodium hydroxide for one hour at room temperature. The control injection of 2 cc. of 1.5 per cent. saline gave a somewhat greater effect than the alkali-treated pituitary tartrate.

to normal sodium hydroxide for one hour at room temperature. It has already been stated that treatment with alkali completely abolishes every known physiological action of the pituitary tartrate and that the above treatment with acid is equally drastic in its effects with the exception that the oxytocic property of the salt is not so completely abolished as in the treatment with alkali.

Antidiuretic action of the tartrate in diabetes insipidus. Von den Velden appears to have been the first to have made the important discovery that the administration of pituitary extracts *sub cutem* to normal individuals as well as to patients suffering from chronic renal disease causes the kidneys of both these classes of individuals to secrete less urine and of higher specific gravity. This author also found that the antidiuretic action of the extract was especially efficacious in a case of diabetes insipidus nervosus. His patient obtained immediate, if only temporary, relief from the distressing symptoms. Coincidentally Farini related the case of a girl aged twenty-three suffering from marked diabetes insipidus who was successfully treated by him with subcutaneous injections of pituitary extracts. The further and very extensive literature bearing on this use of pituitrin in diabetes insipidus, as well as that dealing with the surgical lesions of the gland in relation to experimental polyuria (Cushing and his co-workers) and also that dealing with the relation of clinical disorders of the pituitary to diabetes insipidus, cannot be taken into consideration in this paper. One fact emerges very clearly from the great amount of experimental work that has been done since the appearance of the earlier papers on this subject and that is that the diuretic effect produced by pituitary preparations in experiments on animals is of transient duration and that the really characteristic and more important action of these preparations on normal human beings as well as on those afflicted with diabetes insipidus is antidiuretic. Our own work seems to show that the transient diuretic action of our tartrate on green-fed, urethane-anesthetized rabbits may apparently serve as a means of gauging its antidiuretic activity in diabetes insipidus, though undoubtedly the pressor action of the salt would be a better

index of its antidiuretic action, inasmuch as the lowering of the urinary output seems to depend largely, if not entirely, on the vaso-motor action of the drug.

Through the kindness of Professor L. G. Rowntree of the Mayo clinic, of Dr. A. M. Snell of the Mankato Clinic and Doctors McCann and Keefer of Professor Longcope's clinic and Doctor Bugg of Professor Howland's clinic, we were placed in a position to test our tartrate on four patients suffering from primary diabetes insipidus. The tartrates employed were not of the highest degree of purity. They ranged from 50 to 75 times β -I phosphate in the therapeutic trials at the Mayo Clinic to 160 times β -I phosphate at the Hopkins Hospital trials. All of the clinical and therapeutic data bearing upon these trials are given in detail in a paper by Abel and Geiling and a perusal of that paper will, I think, convince the reader that our tartrate possesses the characteristic antidiuretic property for diabetes insipidus in equal measure with that exhibited by the ordinary commercial pituitary extracts. Although the tartrate used in these clinical trials did not have the highest oxytocic value, as unfortunately none of the more purified salt was at our disposal at the time, I cannot believe that the results would have been any different had the purer tartrate been used, such for example as the one whose oxytocic titer is 500 times β -I phosphate and the diuretic action of which is so well illustrated in Figure 7. The less pure tartrates have been shown in my laboratory to contain as contaminating substances only crystalline salts which are entirely devoid of physiological properties and this is the reason that the only difference which we have found in our work with these tartrates is one not of a qualitative but of a quantitative character. That is to say, the purer the tartrate the less is required of it for the production of any given physiological effect whether it be antidiuretic or oxytocic in its nature. It would appear therefore that our tartrate possesses the antidiuretic property of the posterior lobe extracts to the same extent that it exhibits all of the other characteristic physiological prop-

erties of such extracts—oxytocic, cardiovascular and respiratory—which have been discussed in this lecture.

IS THE POSTERIOR LOBE PRINCIPLE WITH ITS MANIFOLD
PHYSIOLOGICAL ACTIONS A TRUE HORMONE?

Having shown that the principle under discussion is one of extraordinary potency and possessed of a variety of physiological actions, the question that now arises and demands attention is—is this product a true hormone, a principle of internal secretion which finds its way ultimately into the general circulation and from there to various organs? Anatomists and histologists all admit that it is quite possible for an active principle to find its way into the circulation from the posterior lobe either by way of the perivascular lymph channels into the cerebro-spinal fluid and from there into the venous sinuses, or by way of the absorbing blood capillaries directly into the circulation. The histological structure of the pars posterior apparently gives no support to the belief that an active principle is produced in this part of the hypophysis; in a word, there is no positive anatomical evidence that an internal secretion is formed in the posterior lobe itself. The theory that the active principle of the posterior lobe is really derived from the pars intermedia was first advanced by Herring in 1908, on the basis of extensive histological studies of the mammalian pituitary body, and the theory met with favor on the part of Houssay and others. Herring describes groups of cells belonging to the pars intermedia which penetrate the posterior lobe and he suggests that these cells furnish a secretion in the form of colloidal globules or masses which supply the active principle to the pars posterior. This view, however, is not generally accepted. Many critics, as Bremer and Bailey, suspect that this colloidal material does not represent an active product but is merely the result of cell degeneration.

Schmidt and May are often cited as having furnished evidence that an antecedent of the posterior lobe principle is contained in the tethelin of Robertson. E. Leschke quite independently of these authors also makes a similar suggestion and on similar grounds. Schmidt and May found that when tethelin is

treated with barium hydroxide the resultant break-down products cause contraction of the guinea pig's uterus and also induce a small rise of blood pressure. From these observations they conclude that the active principle of the posterior lobe finds its chemical precursor in the anterior lobe. Now tethelin as we have seen is not a chemical individual and arguments derived from such experiments as those made by Schmidt and May and by Leschke have to my mind no necessary bearing whatever on this question. The split products of all sorts of tissue elements which are in no way related to the pituitary principle easily give a small effect on the guinea pig's uterus.

One is not justified in taking it for granted that the principle under discussion cannot arise in the posterior lobe itself. A substance of such extraordinary potency need only be produced in very minute quantities even in times of emergency. As Cowdry remarks in another connection the absence of visible secretion antecedents in the cells of a structure may mean very little, for he points out that an active and necessary constituent is produced in the anterior lobe of young tadpoles, for example, in an early stage before evidence of cell differentiation makes its appearance. While the experiment neither strengthens nor weakens the supposition that the neural tissue of the posterior lobe cannot or does not itself produce an active principle like the one under discussion, it may be stated in this connection that Dr. Geiling and I recently made extracts from a gliomatous tumor immediately after its removal from the skull of a patient by Dr. Dandy and found that this extract gave no evidence of containing the blood pressure-raising principle. The only obtainable effect on the blood pressure was the marked depressor action which is so characteristic of extracts of cerebral tissue.

Professor Dixon of Cambridge has within a few months past made the claim, based on very convincing experiments of his own, that the cerebro-spinal fluid of dogs always contains a small amount of plain muscle (uterine) stimulating substance which he believes to be derived from the posterior lobe. He finds that after total extirpation of the hypophysis, some of this

principle is still contained in the cerebro-spinal fluid. When, however, an extract of the posterior lobe is injected intravenously into a normal animal, he discovers that the cerebro-spinal fluid contains a greatly increased amount of the uterine stimulant. He concludes that this enrichment is not due to a direct passage of the injected posterior lobe principle from the blood vessels into the cerebro-spinal fluid but that it is due to the fact that the injected active principle stimulates the hypophysis to secrete an increased amount of the principle. This action, as Dixon says, would be perfectly analogous to that which is seen when an injection of bile salts causes the liver to secrete more bile and more bile salts.

Certain earlier observers have obtained results with cerebro-spinal liquor which are not in agreement with those of Dixon. Thus, C. and M. Oehme state that with the normal, unconcentrated cerebro-spinal fluid of laboratory animals they could obtain no distinct evidence of any action on the uterus of the guinea pig. They consider the uterine test as one that is not sufficiently sensitive for the detection of an oxytocic stimulant in this fluid, as it was only after the addition of as much as 10 c.c. of the cerebro-spinal fluid to the uterine bath containing 60 to 80 c.c. of Ringer's solution that an occasional minimal reaction could be obtained. It should be stated, however, that while these authors could obtain no satisfactory evidence of the existence of an oxytocic principle in normal cerebro-spinal liquor they nevertheless claim that a vaso-constricting substance or substances are present in this fluid. They rest their claim on perfusion experiments with the surviving rabbit ear (Krawkow-Pissemsky method), in which a very marked diminution in the outflow was observed when centrifugalized lumbar-puncture fluid was employed as the perfusion liquid. The observed vaso-constricting action was surprisingly large, being equal to that obtained with a commercial pituitary extract when diluted 1:10,000 to 1:20,000. It need hardly be pointed out that this extraordinary observation cannot be brought into agreement at the moment with the experiments of others in regard to the

hemodynamic action of cerebro-spinal liquor. It should also be noted that the Oehmes state clearly that their biological tests are inadequate for the identification of the assumed vaso-constricting principle.

Leschke must also be named among experimenters who state that they have never obtained any evidence of the existence of an oxytocic principle in cerebro-spinal fluid. Dixon states that the cerebro-spinal liquor varies greatly in its content of the oxytocic principle. He has seen as little as 10 drops of normal cerebro-spinal fluid produce a decided contraction of the uterus but finds that the usual amount required to elicit an effect is 3 or 4 c.c., and occasionally even larger amounts are inactive. May it not be, then, that the negative results of the Oehmes and of Leschke in respect to the oxytocic evaluation of cerebro-spinal fluid are explainable on the assumption that, making fewer observations than Dixon, these authors happened upon a larger percentage of cerebro-spinal fluids which contained only very little of the uterine stimulant?

Trendelenburg has recently confirmed Dixon's results, with the exception, however, of finding that 1 c.c. of the cerebro-spinal fluid of cats corresponds in its action on the surviving uterus of rats and guinea pigs to only 1/35,000 mgm. of fresh posterior lobe tissue as against Dixon's highest oxytocic evaluation of 0.2 to 2 mgm. for 1 c.c. of the cerebro-spinal fluid of the dog. Trendelenburg finds that the cerebro-spinal fluid of cats produces contractions of the guinea pig's uterus even when diluted five-fold and that its activity is very much decreased twenty-four hours after transecting the stalk of the pituitary gland.

It would appear, then, that cerebro-spinal fluid of animals contains an oxytocic principle, but before it can be definitely accepted that this oxytocic principle is identical with that found in the pars intermedia and posterior lobe of the hypophysis, additional experimental evidence must be adduced. This is absolutely necessary in view of our demonstration that the posterior lobe contains only one specific hormone which has blood

pressure raising, antidiuretic, respiratory and other physiological actions in addition to its oxytocic action.

Now, in respect to the hemodynamic action of normal cerebro-spinal fluid there has been much controversy. The latest paper on this subject is that of C. Jacobson, which also takes into account the work of earlier experimenters (Cushing and Goetsch, Carlson and Martin). This author concludes, on the basis of a large number of blood pressure experiments made with human and bovine cerebro-spinal fluids, that "the effect is essentially a depressor one instead of a pressor one indicative of the posterior lobe secretion."

The only experimenter who, as far as I am aware, has studied the effect of the cerebro-spinal fluid on the function of the kidneys is E. Leschke. This author states that he has not been able to obtain the slightest evidence of the presence of an antidiuretic principle in cerebro-spinal fluid after the injection of 20 to 30 c.c. of the fluid into normal human beings who have taken a liter and a half of water early in the morning on an empty stomach. This negative result need occasion no surprise when it is reflected that one could hardly expect an antidiuretic effect from the few millionths of a milligram of the active principle which, as I calculate from Trendelenburg's data, is all that would be present in so small a quantity as 20 to 30 c.c. of cerebro-spinal fluid. It would, in my opinion, be necessary to work up much larger quantities of the cerebro-spinal fluid and attempt a rough isolation of the oxytocic principle in order to obtain enough of it to make worth while antidiuretic tests on the human being. I have calculated, on the basis of therapeutic trials with our tartrate, that it would require around 1/100 mgm. of a tartrate with an oxytocic titer of 1250, administered subcutaneously, to give in a case of human diabetes insipidus the usual antidiuretic effect lasting three or four hours.

Much, therefore, as I should like to accept without reservation the conclusion of Dixon, fortified, as it now is, by the corroborative experiments of Trendelenburg, I must nevertheless point out that its correctness will not remain unchallenged as long as it

has not been demonstrated that the above named physiological actions of the posterior lobe hormone are exhibited by the cerebro-spinal fluid in addition to its oxytocic action.

In 1915 Weed and Cushing observed that intravenous injection of a post-pituitary extract into dogs, cats and rabbits increased the outflow of cerebro-spinal fluid from a calibrated catheter introduced into the third ventricle or into the subarachnoid cistern. Dixon and Halliburton contend that the pituitary extract induced marked contraction of the bronchi with the consequent appearance of asphyxia and to this abnormal state of the animals in the experiments of Weed and Cushing, the increased outflow of cerebro-spinal fluid is ascribed rather than to an increased fluid production by the choroid plexuses as a result of a stimulating action of the pituitary principle. For a full discussion of the present status of this controversy and of the more recent publications on the action of certain well-known pharmacological agents and of tissue extracts on the cerebro-spinal fluid pressure, I would refer to Weed's comprehensive review on the cerebro-spinal fluid in *Physiological Reviews* for 1922.

As it has not been demonstrated to a certainty that the pars intermedia and the posterior lobe secrete into the cerebro-spinal fluids, may it not be worth while to consider whether the active principle does not find its way directly into the blood stream by way of the capillaries of the posterior lobe? With a view to testing this hypothesis my associates, Drs. Dandy and Geiling, and I, are at the moment engaged in comparing the oxytocic and pressor values of blood drawn from the sinus intercavernosus of dogs (the sinus which receives the venous blood of the hypophysis) with that drawn at the same time from a peripheral vein. At the time of writing no conclusive data have been obtained as we have found that the bloods thus far drawn are highly toxic for the surviving uterus, presumably because of their high content in ether, this being the anesthetic employed by our colleague Dandy in the performance of the surgical operation. To obviate this complication we are now allowing the animals to recover

from the trephining operation and we purpose some days later to abstract blood from the sinus intercavernosus and from the peripheral vein without the use of any anesthetic whatever—a procedure which we regard as quite feasible when undertaken with docile animals. It is also possible that the active principle under discussion finds its way in part into the cerebro-spinal fluid and in part into the blood capillaries of the posterior lobe.

What evidence has experimental surgery to offer in favor of the view that the posterior lobe principle acts as a hormone in the higher animals, quite aside from the manner in which the principle finds its way into the blood? Without going into details it may be said that practically all experimenters agree in stating that the posterior lobe can be removed in the higher animals, as dogs, for example, without danger to life, without, indeed, inducing symptoms of any kind, providing only that no injury has been done to the contiguous parts of the floor of the third ventricle. In the case of the lower animals, however, as in the amphibia, very different and quite unexpected results have been obtained in recent years. A large number of workers, to name only Adler, Gudernatsch, P. E. Smith, Allen, Atwell, Swingle, Hogben and Winton, Pendé and Uhlenhuth, have shown conclusively that one or both lobes of the hypophysis may be removed completely either from adult amphibia or from the larval forms without injury to the contiguous portions of the brain. Leaving out of consideration the results of ablation of the anterior lobe, the concordant experiments of all these investigators prove that the posterior lobe provides an internal secretion in the amphibia which plays a decisive rôle in controlling the pigmentary responses of the skin. Adult frogs (or tadpoles) remain pale after complete hypophysectomy or after removal of the posterior lobe alone, the tadpoles in such cases being termed albinous (P. E. Smith). These pale frogs or albinic tadpoles serve as an extremely delicate indicator for the post-pituitary secretion. Hogben and Winton, in extensive observations some of which were in confirmation of the findings of workers in this country, note that the injection of extracts of the pars intermedia-pars

posterior of the pituitary gland of mammals, birds, reptiles, amphibia and fishes causes the melanophores of hypophysectomized pale frogs to expand to a maximum in about twenty minutes to an half and hour, thus restoring their dark appearance. Addition of an ordinary commercial posterior lobe extract to the water in which albinous tadpoles are swimming also soon causes them to assume the deeply pigmented appearance of normal tadpoles. Through the kindness of Doctor Swett of the Anatomical Institute of this University we were put in possession of a number of such hypophysectomized or albinous tadpoles and it was easy for us to show that the addition of a minute amount of our tartrate to the medium in which the tadpoles were living caused their melanophores to expand as promptly and effectively as did the addition of an aqueous extract of the posterior lobe.

A beautiful and striking experiment showing this expansion of the melanophores is the following:—A frog is placed in a white porcelain receptacle covered with a glass plate and exposed to a strong light for six or more hours, as advised by Hogben and Winton, until it turns into a pale frog, or one whose melanophores are contracted to an extreme degree (pale frog). A pale frog of this sort is decapitated, both hind legs are ligatured just below the knee-joint and then severed from the body. A drop or two of a saline solution of pituitary tartrate with an oxytocic titer of 160 times β -I phosphate and containing 0.016 mg. per 0.1 c.c. is injected underneath the skin close to the plantar surface of the foot near the cruro-tarsal joint and by gentle manipulation the fluid is worked down into the lymph sacs surrounding the toes. The web of the foot is now expanded over a pledget of cotton, placed on a large watch glass and examined under a low power of the microscope. The other leg is injected with an equal volume of normal saline at the same site as mentioned above, and this is examined under a second microscope. Corresponding areas of the skin over the toes of the two feet, or the web of the feet are selected for comparison. Before injection the skin melanophores of both feet are, generally speaking, seen only as small circular black spots, but in a few

minutes the most striking difference is observed; the melanophores of the foot which has been injected with the pituitary tartrate begin to expand in a "reticulate" or stellate manner. The expansion attains a maximum in about 20 minutes. The foot injected with saline shows no change whatever, the melanophores having remained in their previous state of extreme contraction. The difference between the two feet is also visible to the naked eye, the skin of the foot injected with pituitary tartrate being much darker. These experiments were carried out at a room temperature of 84° F. The effect on the melanophores of the frog's foot in the above experiment was so rapidly obtained and was so very pronounced that there can be no doubt that the solution was needlessly strong, and that marked expansion of the melanophores would be equally well obtained with much weaker solutions.*

It may be stated in this connection that I am fully aware that most experimental zoologists refer the production of the melanophore-expanding agent in the amphibia to the *pars intermedia*. There is, however, a sharp difference of opinion among them when it comes to the action of the bovine post-pituitary extracts employed in medicine which are in reality extracts of the *pars intermedia* plus *pars posterior*. P. E. Smith, for example, states in a footnote that the immersion of larvæ in pituitrin (Parke, Davis and Co.) "has produced neither constant nor pronounced changes in the pigmentary system, even when used in strengths sufficient to cause the serious distress or death of animals." Hogben and Winton, on the other hand, invariably obtained expansion of melanophores under all circumstances with very small doses of the liquid sterile posterior

* These preliminary experiments on hypophysectomized tadpoles and on the frog's foot were made only with relatively impure preparations of our tartrate and whether the action on the melanophores was due to the pressor-oxytocic-diuretic principle or to an admixed impurity will have to be determined by using the most highly purified salt. It also still remains to be determined if the melanophore-expanding action is destroyed by acids and alkalies as are the other physiological properties of our principle.

lobe extract "Infundin" (Burroughs, Wellcome). In view of their results Hogben and Winton state that they are able "for the first time to bring the results of hypophysectomy into harmonious relation with the effects of pituitary administration." May not the negative results of Smith be due to the chloretone which is present in the pituitrin of Parke, Davis and Co.? Both of the commercial preparations here named are extracts of the pars intermedia plus pars posterior, as already stated. It would seem that the positive results of Hogben and Winton, based on an extensive and well-controlled series of experiments, are the more acceptable for the present. These authors appear to take it for granted that it is the pars intermedia of the pituitary gland of the amphibia which has the specific action on the melanophores. I find no record of any experiments made by them in which the melanophore-expanding agent is localized either in the pars intermedia or pars posterior or both in the higher animals. Smith, in the footnote referred to above, says "an emulsion of the posterior lobe (less the pars intermedia), even when used in strengths greatly in excess of that employed with the pars intermedia, appears not to evoke this reaction."

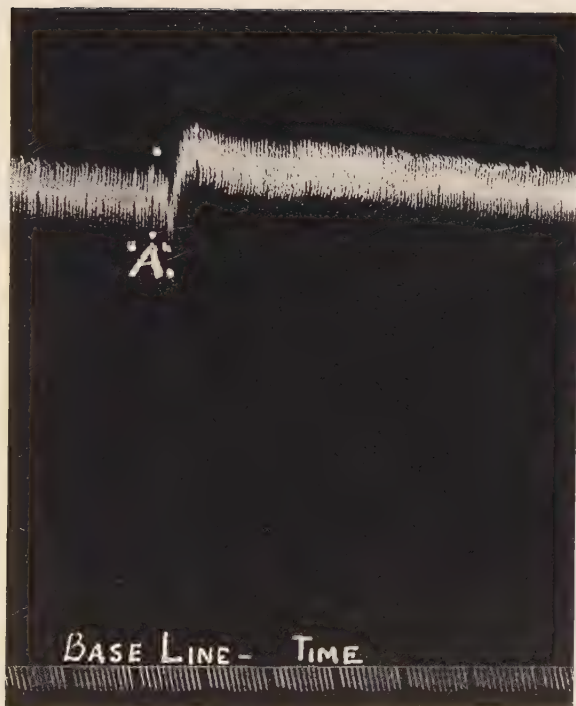
There are no doubt other functions of the posterior lobe in the amphibia which are as yet unknown and the absence of which, like that seen when the specific local action on the melanophores is abolished, is not incompatible with a continued existence. At any rate, we find in these experiments on amphibia and in this striking example of substitution therapy by our tartrate as well as by post-pituitary extracts clear proof that in these animals the posterior lobe secretes a true hormone into the blood which exerts an influence on cellular structures which are far removed from the hypophysis.

Are we to conclude from the negative results of ablation of the posterior lobe in the higher animals that this part of the hypophysis (inclusive of the pars intermedia) plays no rôle whatever in the economy of these animals, that it is entirely without any physiological significance? To draw such a conclusion from these experiments is entirely unjustifiable. I am quite prepared

to believe that the entire hypophysis can be removed from the adult dog without inducing permanent alteration of any kind in the animal, but I am not prepared to admit that an operation of this nature results in the removal of every remnant of tissue exhibiting hypophyseal functions. Let us confine ourselves for a moment to a consideration of posterior lobe ablation alone. It is easy to demonstrate that the ordinary so-called complete extirpation of the hypophysis or of the posterior lobe in the higher animals fail entirely to remove all tissue that has the pharmacodynamic properties of the posterior lobe. Doctor Geiling and I have repeatedly made the following experiment, full details of which will soon be published. We have removed the entire brain of the dog and of the sheep immediately after death, transecting the hypophyseal stalk carefully and with the employment of as little traction as possible. The brains are then placed bottom upward, the cut hypophyseal stalk is carefully elevated with pincers and then transected with small sharp scissors so close to the brain that a depression is left in the base of the brain with an opening into the third ventricle. In a word, the stalk is removed together with some of the contiguous tissue comprising the floor of the third ventricle. Dr. Dandy, who has personally observed these manipulations, assures us that in no instance of experimental hypophysectomy does the surgeon so completely remove the upper portion of the hypophyseal stalk.

A half dozen sheep brains are prepared in this manner, the heads of the animals having been brought still warm from a nearby slaughter house. On the lower surface of each brain at the former site of attachment of the hypophyseal stalk there is now a small cone-shaped depression opening into the third ventricle. We have been mindful of the danger of contaminating the cut surfaces with neural tissue which might possibly be squeezed out of the inner part of the hypophyseal stalk and have for these reasons avoided traction on the stalk and have even excised along with it a small portion of the adjoining hypothalamus, as already stated.

The next step consists in the careful removal of the tissue



SHOWS THE EFFECT ON THE BLOOD PRESSURE OF A CAT OF AN EXTRACT
MADE FROM THE FLOOR OF THE THIRD VENTRICLE OF A
SHEEP BRAINS.

FIG. 10.—Cat, male, weight 2.9 kg. Ether anesthesia. "A." Injection of 0.5 cc. extract
(0.03 gm. tissue). Time in five second intervals.

immediately surrounding the site of the upper end of the removed hypophyseal stalk. Anteriorly, in this dissection, some of the soft tissue covered by the optic chiasma is removed and posteriorly a part, if not all, of the tuber cinereum is included. The amount of brain tissue thus dissected out from six prepared sheep's brains varies from 0.2 to 0.4 gram. It is immediately ground up with a few c.c. of a 0.25 per cent. solution of acetic acid, brought to a boil and filtered. The intravenous administration of one-half of a cubic centimeter of this extract, representing only 0.03 gram of hypothalamus tissue, induces a distinct rise of the arterial pressure in an anesthetized cat as may be seen from Figure 10. Naturally, the injection of somewhat larger quantities gives a correspondingly increased effect on the blood pressure. This extract also stimulates the guinea pig's uterus in a manner characteristic of post-pituitary extracts. We may regard it as demonstrated, therefore, that the tissue of the hypothalamus continuous with the upper end of the hypophyseal stalk contains the posterior lobe hormone. At the moment we are not able to state how this tissue compares in respect to its content of hormone with the posterior lobe itself.

As is well known, extracts of brain tissue in general contain a powerful blood pressure-lowering agent, with absolutely no indication of the presence of a pressor substance. We have in all cases matched our pressor extracts of tissue from the floor of the third ventricle with similar depressant extracts made from the tissues lining the lateral ventricles. These extracts were very active in lowering the arterial pressure and were found to have only a feeble oxytocic action in comparison with that of the former.

Extracts were also made of the posterior part of the hypothalamus inclusive of the corpora mammillaria, and the blood pressure tracings obtained after the intravenous injection of such extracts show them to contain considerably less of the pressor-oxytocic body and much more depressor substance than were found in extracts made from the anterior division of the hypothalamus.

The demonstration by Geiling and myself that there exists in the grey matter in the floor of the third ventricle a tissue which yields an extract possessed of pressor and oxytocic properties, or in other words a tissue capable of elaborating a principle which appears to be identical with that found in the pars posterior and pars intermedia of the hypophysis, supplies me with the necessary data for putting forward an explanation entirely different from that offered by Camus and Roussy for their findings. If these investigators in their experimental hypophysectomies did not remove all of the specific hormone-producing tissue in the anterior hypothalamus—and such was certainly the case in view of our above-named observations—it is not surprising that no polyuria or other signs of posterior lobe deficiency were noted by them. In all so-called total hypophysectomies hitherto performed in adult animals and which were followed by no observable symptoms, the amount of hormone-producing tissue, although constituting only a small fraction of the total posterior lobe material, seems, nevertheless, to have sufficed for the production of the necessary physiological minimum quantity of an antidiabetic substance.

Camus and Roussy, as also Bailey and Bremer, found that puncturing the grey matter in the neighborhood of the tuber cinereum was promptly followed by polyuria in hypophysectomized animals which had not previously shown any symptoms. In experiments of this kind is it not reasonable to suppose that such a puncture (of the floor of the third ventricle in such previously hypophysectomized animals) injured the last remaining portion of hormone-yielding tissues situated in the hypothalamus? Such an explanation of the results obtained by the aforementioned investigators naturally involves the assumption that the posterior lobe and pars intermedia, and the similarly functioning tissue of the grey matter in the hypothalamus, elaborate a hormone which when present in insufficient amount leads to the production of polyuria or diabetes insipidus. This pathological condition would on this theory find its explanation in a deficiency of a specific hormone which controls the removal of water and

certain electrolytes from the body via the kidneys. Such a theory is quite in opposition to that of Camus and his collaborators and others who explain the polyuria incident to the puncture of the hypothalamus in hypophysectomized animals on the assumption of injury to *hypothalamic nerve centers*. These observers further claim that puncture of the hypothalamus is equally effective in producing polyuria *in animals whose hypophysis has remained entirely untouched*. One would suppose that in this case the apparently uninjured pars intermedia and pars posterior could furnish to the body the necessary amount of the specific hormone needed by it, and hence that the destruction only of the upper and much smaller area of hormone-yielding tissue could not induce polyuria. Before accepting such an explanation it would have to be shown that puncture of the hypothalamus in the immediate neighborhood of the upper attachment of the hypophyseal stalk in no wise interferes with either the production of the hormone in the pars intermedia or pars posterior, or with its transference to the cerebro-spinal fluid or to the blood stream or to both. It is a curious fact that in frogs a puncture of the brain, trifling in itself, and followed by no observable symptoms can nevertheless be shown to have caused certain profound alterations in the response of the frog's brain and spinal cord to a whole series of sulphonated convulsant dyes. In past years my collaborators (Syz and others) and I have found that various trifling experimental lesions of the frog's brain cause this organ to take up such dyes in great abundance, and convulsions are produced in these animals by doses very much smaller than would be required to produce the same symptoms in normal animals. Furthermore such frogs differ from normal ones in that their brain and spinal cord take and retain a great deal of the injected dyes. It is therefore within the range of possibility that puncture of the hypothalamus is not without an effect on the functional integrity of the pars posterior and pars intermedia.

Dott reports that the placing of a small piece of platinum, so that it rests on and irritates the pars intermedia and pars posterior, gives rise in the dog to an abundant polyuria—1200 to

1500 per cent. above the normal. He explains this pathological condition by assuming it to be due to an increased secretion of the pars intermedia resulting from a lasting irritative stimulation of that region. Could it not as well be assumed that the effect of the continuous irritation interferes with the production or elimination of the necessary antidiuretic hormone, resulting in a condition of hypo-function?

Perhaps experimental polyuria would follow puncture of the posterior lobe without injury to the hypophysis. Certainly a temporary diabetes insipidus is frequently described as being incident to the extirpation of the gland. It is within the bounds of possibility also that an injury of the upper or hypothalamic portion of the neural hypophyseal tissue interferes more markedly with its functions, for reasons broached above, than one of the pars posterior.

I have thrown out the above suggestions because the underlying assumption is in agreement with the known facts of physiology. A small part of the liver, a fraction of the insular tissue of the pancreas (one-fifth), one parathyroid glandule out of four, may, in each instance, suffice to carry on the functions of the gland, although four-fifths or more of the glandular tissue in question may have been destroyed by disease or removed in an experiment.

To recapitulate, I feel confident that further research will demonstrate as satisfactorily for the higher animals as for the amphibia that the posterior lobe with the pars intermedia and the similarly functioning tissue of the hypothalamus elaborates a specific hormone which controls the removal of water from the body via the kidneys and perhaps other organs. Is it an entirely untenable supposition that a deficiency of this hormone should lead to a polyuria or diabetes insipidus?

This theory finds strong support in the opinion of clinicians like Frank and Marañón who have long maintained (Frank since 1910) that there is a causal connection between morbid states of the hypophysis and diabetes insipidus and who therefore class the posterior lobe (or some other division of the hypophysis con-

cerned with the movement of water in the body) with the internal secretory organs. In opposition to these writers other clinical authorities, Aschner, Leschke and others, have brought forward many facts, both clinical and experimental, which in their opinion show that lesions of the hypothalamic centers are essential, in part at least, in the causation of this disease.

We may ask, in conclusion, what proof we have that the anterior lobe of the hypophysis has any significance for the higher animals and human beings. It has been maintained in the past by a few authorities, such as the clinician, Strümpel, that the gland is a vestigial structure without any present physiological significance for the higher animals. Camus and his collaborators have already been cited as refusing to believe that the posterior lobe is of functional significance. Their skepticism also extends to the anterior lobe. These investigators would set aside the well known clinical entities, gigantism, acromegaly, dystrophia adiposogenitalis, the hypophyseal cachexia of Simmonds and diabetes insipidus as having no connection whatever with the hypophysis. They believe that all these diseases can be correlated with morbid alterations or with experimentally induced lesions of the hypothalamus. I cannot here enter into the literature of this subject in detail or name those who have given either a partial or their entire support to such views. Bailey has given a careful critical review of the earlier experimental work of Paulesco, Horsley, Cushing and many others, the perusal of which leaves one with the impression that the enormous amount of experimental work that has been done on the higher animals (as the dog for example) has not led to decisive results in this controversy. In regard to acromegaly, for example, Bailey says that he would not be cited as a defender of the theory that acromegaly is caused by a lesion of the hypothalamus, but thinks that this assumption does not lie outside the bounds of possibility. He believes that the adiposogenital syndrome when experimentally produced is caused by injury of the hypothalamic nuclei, but states that this view cannot be definitely accepted until some one succeeds in removing the hypo-

physis without producing this syndrome and then produces it in the same animal by an experimental lesion of the hypothalamus.

It is also not possible for me to cite in this connection at any length the writings of numerous continental clinicians and experimentalists but my reading shows me that among them are many men of wide clinical experience who hold that most, if not all, of the clinical states cited above, with the possible exception of Simmonds' hypophyseal cachexia, must not be regarded as being associated with lesions of the hypophysis alone but jointly with lesions of important nerve centers of the hypothalamus and of the hypophysis. In the minds of these writers the neural or hypothalamic nerve center involvement outweighs the endocrine function of the anterior lobe as a causal factor in the explanation of the so-called hypophyseal states.

If I may be permitted to have an opinion of my own in regard to the relationship of the anterior lobe of the hypophysis to the diseased conditions cited above, and to the lesions experimentally induced by surgeons, I would offer a suggestion along the lines made in regard to the posterior lobe. Is it not possible that the pars tuberalis and a portion of the hypothalamus immediately contiguous with the upper part of the hypophyseal stalk possess, if only to a limited extent, the well known growth-promoting hormone of the anterior lobe? My collaborators and I have not yet had the opportunity to test this hypothesis in an experimental way, as was done by us for the posterior lobe hormone. The pars tuberalis was shown by Atwell and Marinus to contain none of the posterior lobe hormone, but it is nevertheless possible that it contains the growth-promoting hormone. If tissue producing this hormone extends even a small way into the hypothalamus, then this fact will perhaps serve to coördinate lesions of the hypothalamus and the hypophysis and bring both into harmony with the view that we are dealing in these clinical and experimental conditions rather with a hormone function of the anterior lobe and the upward-extending tissue than with lesions of hypothalamic nerve centers, however important these

latter may be as neural regulating mechanisms comparable to others of like nature located elsewhere in the brain.

The experimental zoologists referred to earlier in this lecture have shown that the anterior lobe of the hypophysis, in the case of the amphibia, secretes a hormone which has growth-promoting properties, and that a "replacement" therapy is applicable in these animals in conditions of hypo-function due to lesions of or removal of this lobe. It may be freely admitted that it has not been proved with equal certainty that the anterior lobe plays a similar rôle in mammals. It is, however, my personal view, which I find is shared by others, that it only needs further continued experimentation, coupled with histological and clinical studies, to establish the theory that the anterior lobe secretes a growth-promoting hormone (possessed doubtless of other properties also) on as firm a foundation as has been done for the amphibia. As matters stand, the theory of the endocrine function of the anterior lobe of the hypophysis in the higher animals finds greater support, both in the experimental and clinical knowledge of the day, than does the corresponding theory of the endocrine function of the posterior lobe, but as I have already intimated I am also optimistic in believing that the ultimate proof of the correctness of the latter hypothesis will soon be forthcoming.

LITERATURE

- Abel and Kubota: Jour. Pharm. and Exp. Therap., 1919, xiii, 243.
Abel and Nagayama: Jour. Pharm. and Exp. Therap., 1920, xv, 347.
Abel and Rouiller: Jour. Pharm. and Exp. Therap., 1922, xx, 65.
Abel, Rouiller and Geiling: Jour. Pharm. and Exp. Therap., 1923, xxii, 289.
Abel and Geiling: Jour. Pharm. and Exp. Therap., 1923, xxii, 317.
Addison: Lond. Med. Gaz., 1849, xliii, 517, and "On the Constitutional and Local Effects of Disease of the Suprarenal Capsules." London. 1855, S. Highley.
Adler: Arch. f. entwick. Mech., 1914, xxxix, 21.
Allen: Anat. Rec., 1917, xi, 486.
Biol. Bull., 1917, xxxii, 117.
Science, N. S., 1920, lii, 275.
Aschner: Pflüger's Arch. f. d. ges. Physiol., 1912, cxlvi, 1.
Wien. klin. Woch., 1912, xxv, 1042.

- Atwell: *Science*, N. S., 1919, xlix, 48.
- Atwell and Marinus: *Amer. Jour. Physiol.*, 1918-19, xlvii, 76.
- Bailey: *Erg. d. Physiol.*, 1922, xx, 162.
- Banting, Best, Collip, Campbell and Fletcher: *Can. Med. Assn. Jour.*, N. S. 1922, xii, 141.
- Basedow: *Woch. f. d. ges. Heilk.*, Berlin, 1840, viii, 197, 220.
- Bernard: *Comp. rend. Acad. d. Sci. Par.*, 1857, xlv, 578.
- Bert, quoted by Gley: "The Practitioner," January, 1915.
- Berthold: *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1849, 42.
- Biedl: *Innere Sekretion*, Berlin, 1913.
- Bremer: *Ann. et Bull. d. l. Soc. Roy. d. Sci. Méd. et Nat. de Bruxelles*, 1923, viii, 129.
- Brown-Séquard: *Comp. rend. Acad. d. Sci. Par.*, 1856, xliii, 422, 542.
Arch. de physiol. norm. et path. Par., 1889, 5 s., i, 651, 739.
- Burn and Dale: *Res. Council London Repts. on Biol. Standards*. 1: On the Physiol. Standardization of Ext. Post. Lobe of the Pit. Body, 1922, p. 38.
- Camus and Roussy: *Titres et Traveux Scientifiques du Dr. J. Camus*, 1921, Paris, Imprimerie Chaix.
- Carlson and Martin: *Amer. Jour. Physiol.*, 1911, xxix, 64.
- Cowdry: *Endocrinology and Metabolism*, Appleton, 1922, i, 705.
- Cushing: *The Pituitary and its Disorders*, Lippincott, 1912.
- Cushing and Goetsch: *Amer. Jour. Physiol.*, 1910, xxvii, 60.
- Cybulski: *Gazeta lekarská*, 1895, No. 12; *Centralblatt f. Physiol.*, 1895, ix, 172.
- Cyon: *Archiv. f. d. ges. Physiol.*, 1898, lxxi, 431; 1898, lxxiii, 339; 1900, lxxxi, 267.
- Dale: *Biochem. J.*, 1909, iv, 427.
- Dixon: *J. Physiol.*, 1923, lvii, 129.
- Dixon and Halliburton: *J. Physiol.*, 1916, 1, 198.
- Dott: *Quart. Jour. Exp. Physiol.*, 1923, xiii, 241.
- Dudley: *J. Pharm. and Exp. Therap.*, 1919, xiv, 295.
- Engeland and Kutscher: *Zeit. f. Biol.*, 1912, lvii, 527.
- Erdheim: *Beitr. z. path. Anat. u. z. allg. Path.*, 1903, xxxiii, 158.
- Evans and Long: *Anat. Record*, 1922, xxiii, 19.
- Farini: *Gazz. degli Osped.*, Sept. 11, 1913, cited from *Epitome*, p. 76, *British Med. J.*, Nov. 29, 1913.
- Fontani, quoted by Hyrtl: *Lehrbuch d. Anatomie*, 1884, p. 878.
- Foderà and Pittau: *Pathologica*, 1909, i, 269.
- Frank: *Klin. Woch.*, 1924, iii, 847, 895.
- Fühner: *Münch. Med. Woch.*, 1912, 853.
Zeit. f. d. ges. exp. Med., 1913, i, 397.
- Fühner and Pankow: *Arch. f. d. ges. Physiol.*, 1912, cxlvii, 89.

- Galen: "Galen on the Natural Faculties," translation by A. J. Brock, 1916, C. P. Putman's Sons, New York, p. 215.
- Garrison's History of Medicine, 2nd ed., p. 258.
- Gaskell: Quart. Jour. Med. Sci., 1890, xxxi, 379.
- Jour. Physiol., 1889, x, 153.
- Geiling and Kolls: J. Pharm. and Exp. Therap., 1924, xxiii, 1.
- Gley: Compt. rend. soc. biol., Paris, 1891, iii, 551.
- The Practitioner, Jan., 1915.
- Graves: London M. and S. Jour., 1835, vii, 516.
- Guggenheim: Biochem. Zeit., 1914, lxv, 202.
- Gull: Trans. Clin. Soc., London, 1874, vii, 181.
- Hadden: Trans. Clin. Soc., London, 1880-1, xiv, 58.
- Hanke and Koessler: J. Biol. Chem., 1920, xliii, 557.
- Hédon: Diabète pancréatique, Paris, 1898.
- Herring: Quart. Jour. Exp. Physiol., 1908, i, 281; 1914, viii, 254.
- Hogben: Proc. Roy. Soc., 1923, Sér. B, xciv, 204.
- Hogben and Winton: Proc. Roy. Soc., 1922, Ser. B, xciv, 151; 1923, xcv, 15.
- Horsley: Lancet, 1886, i, 3.
- Brit. Med. Jour., 1906, i, 323; ii, 411.
- Houssay: La acción fisiológica de los extractos hipofisarios, 1918, Flaiban, Buenos Aires.
- Howell: J. Exp. Med., 1898, iii, 215, 245.
- Huxley and Hogben: Proc. Roy. Soc., Ser. B, 1922, xciii, 36.
- Hyrtil: Lehrbuch der Anatomie, 17th ed., 1884, p. 892.
- Jackson and Mills: J. Lab. and Clin. Med., 1919, v, 1.
- Jacobson: Johns Hopkins Hosp. Bull., 1920, xxxi, 185.
- Kocher: Verh. d. deutsches Ges. f. Chir., XII Congress, Arch. f. klin. Chir., 1883, xxix, 254.
- Kolls: J. Pharm. and Exp. Therap., 1920, xv, 443.
- Kolls and Geiling: J. Pharm. and Exp. Therap., 1924.
- Lépine: Lyon Méd., 1889, lxii, 621.
- Leschke: Zeit. klin. Med., 1919, lxxxvii, 201.
- Biochem. Zeit., 1919, xcvi, 50.
- Verh. f. innere Med., 1922, XXXIV Kongress, 349.
- Lewis, Miller and Mathews: Arch. Int. Med., 1911, vii, 785.
- Leyko and Sikorski: Medycyny Doświadczalnej i Społecznej, 1923, i, 1.
- Lower: Tractatus de Corde, etc. Cui accessit dissertatio de origine Catarrhi, in qua ostenditur illum non provenire a cerebro, 2nd ed., London, 1670.
- Macleod: Brit. Med. Jour., Nov. 4, 1922.
- Magnus and Schafer: J. Physiol., 1901-2, xxvii, 9.
- Marañón: Bol. soc. españ. de biol., 1911, 120.
- Marie: Rev. Méd., Paris, 1886, vi, 297.

- Mering and Minkowski: *Arch. f. exp. Path. u. Pharm.*, 1889, xxvi, 371.
- Minkowski: *Berl. klin. Woch.*, 1887, xxiv, 371.
- Moebius: *Centralbl. f. Nervenh. u. Psychiat.*, 1886, ix, 356, 358.
- Nothnagel's *Spec. Path. u. Therapie*, 1896, xxii, 1, 202.
- Monardes: "Historia medicinal de las cosas que se traen de las Indias occidentales que sirven al uso de Medicina, 1565," translated into German from the Latin translation of Clusius, 1579, by K. Stünzner, 1895, Halle a. S., Verlag von Max Niemeyer.
- Mummery and Symes: *Brit. Med. Jour.*, 1908, ii, 786.
- Murray: *Brit. Med. Jour.*, 1891, ii, 796.
- Nagayama: *J. Pharm. and Exp. Therap.*, 1920, xv, 401.
- Oehme and Oehme: *Deutsch. Archiv. f. klin. Med.*, 1918, cxxvii, 261.
- Oliver and Schafer: *Jour. Physiol. (Proceedings)*, 1894, xvi, p. i; 1894-5, xvii, p. ix.
- Oliver and Schafer: *Jour. Physiol.*, 1895, xviii, 277.
- Ord: *Med. Chir. Trans.*, London, 1878, lxi, 57.
- Osborne and Vincent: *Brit. Med. J.*, 1900, Mar. 3.
- Paulesco: *L'hypophyse du cerveau. I. Physiologie*, Paris, 1908, Vigot Frères.
- Pendé: *Endocrinologia*, Milano, 2nd ed., 1920.
- Reverdin and Reverdin: *Rev. méd. de la Suisse romande*, Genève, 1883, iii, 169, 233, 309.
- Robertson: *J. Biol. Chem.*, 1916, xxiv, 397.
- Robin: quoted from Gley.
- Roca: *J. Pharm. and Exp. Therap.*, 1921, xviii, 1.
- Salvioli and Carraro: *Arch. Scienz. med.*, 1907, xxxi, 249.
- Arch. ital. de biol.*, 1908, xlix, 1.
- Sandström: abstracted in Hofmann-Schwalbe's *Jahresb. ü. d. Fortschritte d. Anat. u. Physiol.*, 1880, ix, 1.
- Schafer and Herring: *Proc. Roy. Soc.*, London, 1906, lxxvii, 571.
- Schiff: *Untersuchungen über die Zuckerbildung in der Leber*, Würzburg, 1859.
- Schmidt: *J. Lab. and Clin. Med.*, 1917, ii, 711.
- Schmidt and May: *J. Lab. and Clin. Med.*, 1917, ii, 708.
- Smith, M. I., and McClosky: unpublished manuscript.
- Smith, P. E.: *Anat. Rec.*, 1917, xi, 57.
- Univ. Cal. Pub. Physiology*, 1918, v, 11.
- Strümpel: *Deutsch. Zeit. f. Nervenh.*, 1897, xi, 51.
- Swingle: *J. Exp. Zoology*, 1921, xxxiv, 119.
- Syz: *J. Pharm. and Exp. Therap.*, 1923, xxi, 263.
- Szymonowicz: *Arch. f. d. ges. Physiol.*, 1896, lxiv, 97.

- Tilney: Wistar Inst. Memoirs, 1911, No. 2.
- Trendelenburg: Berl. klin. Woch., 1924, iii, 777.
- Uhlenhuth: J. Gen. Physiol., 1920-1, iii, 347.
- Velden, von den: Berl. klin. Woch., 1913, ii, 2083.
- Vesalius: quoted by Cushny.
- Vincent: The Practitioner, Jan. 1915.
- Vulpian: Compt. rend. acad. sci., Paris, 1856, xliii, 663.
Compt. rend. soc. biol., Paris, 1859, 2 ser., v, 11.
- Weed: Physiol. Reviews, 1922, ii, 171.
- Weed and Cushing: Amer. Jour. Physiol., 1915, xxxvi, 77.

THE FUNCTION OF THE ANTERIOR HYPOPHYSIS ¹

DR. HERBERT M. EVANS

Professor of Anatomy, University of California

EXPERIMENTAL surgery of the brain has recently challenged boldly our conceptions of the endocrine functions of the pituitary gland. In a special and memorable session of the French Neurological Society in June, 1922, Camus and Roussy and their fellow workers—whose conceptions are already in part confirmed by Bailey and Bremer—announced that most, if not all, of the features of the so-called hypophyseal syndromes are due to brain lesions of the hypophyseal neighborhood. It is also important to note that their work forms merely the spectacular part of a slowly accumulating mass of evidence for center or centers in the floor of the diencephalon—from which are regulated temperature, metabolism (at least that of water and carbohydrates) and the functional states of some organs, *e.g.*, the gonads. It is difficult to imagine more consequential relations of any bit of nervous territory than that in the neighborhood of the infundibulum—the “tunnel” in the quaint words of Crooke—which is between the “sleepy arteries” and conveys the “thicker brain excrements” to the “kernel of phlegm” or “phlegmatic glandule.” What indeed has the pituitary gland to do with all of this? Will the new nihilism effectually upset all notions of its function? It is because I do not believe that this is true, because other recent and, I believe, fundamental research points unerringly to distinct endocrine functions of the hypophysis that I venture to rehearse here some of the mooted points. In the endeavor to do this, it does not seem to me possible to overlook the newer conceptions of the adjacent area of the brain, even if it seems equally impossible to deny secretory activity to the clump of epithelial cells which forms the anterior portion of the pitui-

¹ Delivered April 26, 1924. Aided by grants from Comm. Research on Sex Problems, Nat. Research Council and Comm. on Scientific Research, American Medical Association.

tary gland. And if in rehearsing this, I appear to give undue prominence to a series of studies carried out by me, or more particularly, by my associates, I beg your indulgence, firstly, on the grounds that after all one is best fitted to deal with that with which he is acquainted and, secondly, on the clear ground of the need—in my opinion—of the presentation of these more strictly biological aspects of a field where profound clinical interests have been uppermost. One may speak at once, I think, of the advance which biological studies have made by assigning it to two fortunate circumstances—one, that in 1916 Smith and Allen independently successfully ablated the anterior hypophysis of frog larvæ without the possibility of brain injury and secured a train of characteristic effects, and, the other, that Evans and Long had the fortune to establish specific endocrine effects (gigantism and sex disturbance) from parenteral dosage of mammals with beef anterior hypophysis after failure in a long series of massive oral administrations. The latter work was completed in May, 1921, before the recent brilliant demonstration of a similar inefficacy of the pancreatic insular hormone by mouth—and on its completion no persuasion was required to induce Smith of my laboratory to test by our method a replacement therapy in hypophysectomized amphibia. The success of these experiments seemed to us to constitute conclusive arguments in favor of endocrine function of the pituitary gland when there began to appear studies (now considerable in number) which assigned the genital and metabolic, if not the growth, disturbances of hypopituitarism to brain lesions or at any rate showed that these lesions alone could provoke the Fröhlich syndrome. Clinical reports are now also to the same effect (Lehrmitte, etc.). The hypophysis is reported as intact. So important is this recognition that Biedl has recently spoken of two types of such disorders—those with primary brain involvement (hereditary) and those with hypophyseal disorders. It is hence essential to recognize the particular conditions obtaining in mammals and, particularly, to inquire into the possible double nature (endocrine and nervous) of this remarkable metabolic

mechanism. It would appear that such effort must employ both of the new tools or methods of study—surgical and therapeutic; hypophyseal defects must be produced in the mammal without brain involvement (this is supremely difficult) and a replacement medication attempted and, conversely, brain lesions with intact hypophysis must be submitted to the same test even though it appears only remotely possible that either the humoral or nervous pathways of the hypophysis are hurt in this way.² If any of the characteristic symptoms after experimental interference in this neighborhood are remedied by a replacement therapy, we have, I believe, solid support for conceptions of hypophyseal function. I hope to convince you that it is possible to show indisputable evidence of a hypophyseal growth hormone, and that other evidence points to endocrine interrelationships of the hypophysis which cannot be explained on the basis of nervous control and hence nervous responsibility for upset to these organs. I refer to the gonads, thyroid and adrenal.

The ancient position of the epithelial hypophysis, *i.e.*, its position in the ancestors of the vertebrates, appears to have been on the under surface of the head and in front of the mouth. Now in the Amniotes the great development of the nervous system and especially of the forebrain and the flexuring of the embryo dependent upon this, displaces this peculiar patch of ectoderm backward in the stomodæum. It is this ectodermic tissue which constitutes the material of the pouch, or pocket, first recognized in 1838 by Heinrich Rathke. In the Amphibia, fortunately, the anlage of the epithelial hypophysis is in its "primitive" position. It is for a considerable time connected with the surface epithelium and lies between the forebrain protruberance and the

² Negative evidence is always admittedly dangerous support and largely ancillary in its usefulness, and I must pass over, consequently, as impossible of analysis for the present, all of those cases of apparently complete elimination of the hypophysis without symptoms. There is evident difficulty of complete histological analysis in large mammals. Pathologists have now given us the clear demonstration that hypophyseal tissue may be strewn at various places along the embryonic route from pharynx to sella, for example, be completely hidden in the sphenoid.

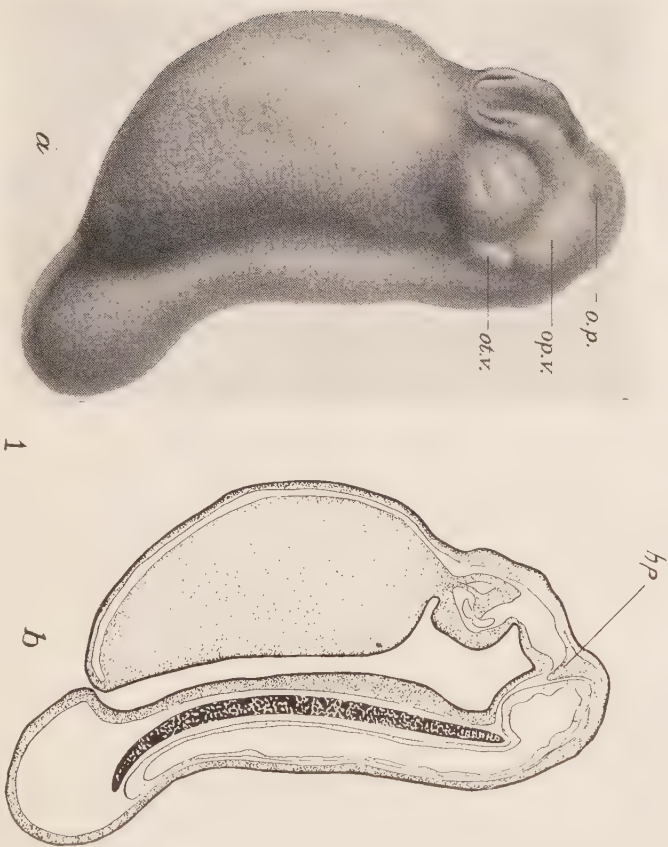


FIG. 1.—Larva of *Rana boylei* four millimeters in length and showing the surface characteristics at the time of ablation of the anterior hypophyseal component. (After Smith). a. Surface. b. Schematic midsagittal view. o.p. Olfactory placode. op.v. Optic vesicle. ot.v. Optic vesicle. hp. Hypophyseal pit.

pharynx. Not only is it at this time accessible from the exterior, but it is not united to the brain, from the ventral surface of which it can be cleanly dislodged. Smith and Allen have shown that the hypophyseal pit can be located and the little lump of tissue cut out of tadpoles which are but three to four millimeters in length and possessing little surface structure, as you can see in figure No. 1, save for a tail bud, adhesive organ, branchial bars, nasal placode and optic and otic vesicles. In 1916¹ these observers simultaneously showed that a striking and characteristic syndrome results from such an operation, a syndrome characterized predominantly by changes in the pigment, growth and endocrine organs of the larvæ. In contrast to their dark, rapidly growing and metamorphosing brethren, these tadpoles acquire a white or silvery look, remain dwarfed, and fail to undergo transformation. Of the remarkable pigmentary changes which convert the tadpoles into silvery and iridescent creatures not unlike trout, I shall have little to say, for analogies with the organs or tissues of mammals are not yet at hand. I would not, however, dismiss this without reference to the satisfactory analysis which can be made of the condition as due to a differential disturbance of the two great groups of pigment bearing cells, those with the black pigment and those with the light, metallic or sheenlike material—the melanophores and the xantholeucophores. The albinos, or hypophysectomized, larvæ, possess a normal number of xantholeucophores which are always widely expanded and a reduced number of melanophores, which are abnormally contracted. Hogben has recently shown that it is possible to secure these color effects in the adult frog by the removal of the hypophysis. Many of the lower vertebrates, particularly fishes, quickly change their color patterns to accord with their environment in a way which has proverbially distinguished the chameleon, and some of these astonishing mechanisms are related to the sense organs, the response hence being participated in by the nervous system. Our main reason for mention of the hypophyseal pigmentary upset is to refer to the clear proof of its direct endocrine or humoral cause, as shown by reciprocal exchange of transplanted

fragments between normal and albinous larvæ. The pigment cells in the transplanted fragments begin to change at once and quickly come to resemble those of the new host, from which tissue juices, of course, diffuse long before nerve filaments can reach the transplant. It is with the growth and other endocrine disturbances of these hypophysectomized larvæ that we must be especially concerned. The albinous larvæ grow slowly, at first not much below that of their controls but as the midlarval period approaches they are noticeably smaller and as the normal larvæ prepare for metamorphosis the albinos may be less than half their size. It is interesting to note that the beef hypophyseal substance as food for this species apparently replaces the growth principle lost by ablation of the epithelial hypophysis, since, as I hope to show later on, I have been unable to increase growth with any amount of oral dosage in normal mammals. We might explain the situation here as due to a different character in the digestive juices of the cold blooded organism. The hypophysectomized larvæ never metamorphose. Since Gudernatsch in 1912 showed that amphibian metamorphosis can be experimentally precipitated by the feeding of mammalian thyroid substance, it was a natural inference that the albinous frog larvæ were defective not only in the pituitary but in their thyroid glands. Now the thyroid could not have been disturbed by the operation, for its neighborhood is far removed from the hypophyseal pit. It was hence of profound interest to find in serial section that the thyroid of albinos was always greatly reduced in size (Fig. 2).

Moreover, thyroid substances, while beginning the metamorphic changes in albinous larvæ, will not bring them to normal completion, so that it is hence probable that both hypophysis and thyroid are essential for these complex events—phenomena, to be sure, for which a true analogue is unknown in the higher animals. It would seem important, however, to fasten attention upon this relationship of thyroid and hypophysis for other reasons than their rôle in amphibian metamorphosis, for the relationship is probably fundamental in nature; the thyroid of the albinous larvæ could not develop because of some necessary stimu-

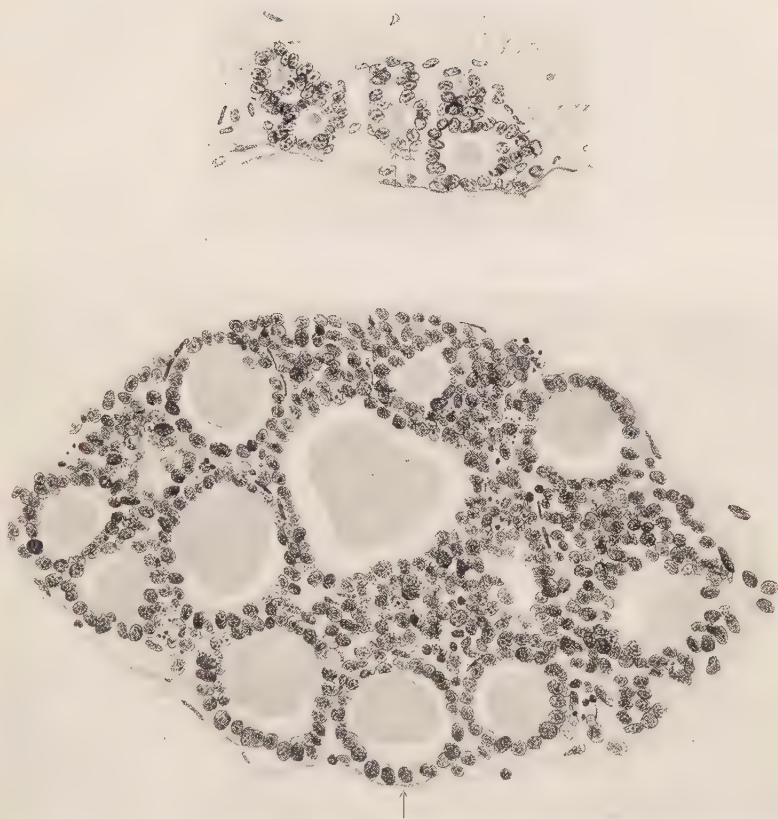


Fig. 2.—Cross sections through the largest portions of the thyroid of an albinus (anterior hypophysectomized) and normal frog larva sixty-four days after the operation. (After Smith).

lus which it should have received from the hypophysis—which had been removed.

What are the laws which govern this relation of thyroid and hypophysis? Knowing now that normal hypophyseal function is essential for thyroid normality, we also feel—as I shall develop presently—that the converse is true and that the thyroid is essential for hypophyseal normality, this being the real cause of dwarfism of cretins. Yet over-function of either of these glands is a disease *sui generis* and does not involve its neighbor; acromegaly is not consequent upon a Basedow, or *vice versa*. In normal life, we have always the adjustment of the hypophysis to the obscure forces which limit normal growth. We are attacking the question of the participation of the sex glands here. While the hypophyseal hormone is thus formed by the utilization of thyroid substances or substances formed by thyroid mediation, it will not overfunction merely from overfunction of the thyroid gland, nor has anyone as yet learned to whip the hypophysis to exceed the forces which control it by nervous stimuli, as were involved in the ingenious experiments of Cannon on the thyroid. We can as yet imitate hypophysis overfunction only by conveying an overabundance of the finished product, or products, of that function—hypophyseal hormones.

What is the evidence of a reciprocal need of the pituitary for the maintenance of thyroid function, the proper explanation of cretinic dwarfism? I feel that hypophyseal function is really reduced after thyroidectomy, though the hypophysis is not smaller and, indeed, undergoes a transitory enlargement (Rogowitsch). Degener has shown that the peculiar enlargement of the anterior hypophysis after thyroidectomy does not represent the assumption of vicarious function. Mammalian experiments which have been made by Smith and Graeser and by Flower and me, show that hypophysectomized animals cannot grow if given thyroid, but that thyroid-free animals possessing the hypophysis will grow normally or better than normally on parenteral administration of the hypophyseal hormone as well as on thyroid therapy. In one case we convey the growth hor-

mone directly, in the other we furnish the animal's own hypophysis with a material needed either directly or indirectly for its normal function.

Other organs are characteristically altered in albinous frog larvæ deprived of their epithelial hypophysis. This is particularly the case with the adrenal cortex, which is reduced without corresponding reduction of the medulla. There is thus a separation in the behavior of the interrenal and chromaffine organs, though the function of the former is entirely obscure to us.

It now seems remarkable to us that though a consensus of opinion assigned acromegalic overgrowth to hyperpituitarism (reversing the opposite conception which Marie had first expressed), yet no one had been able to establish the matter by direct experiment. Indeed, the most recent summaries of our knowledge in this field still refer to the failure of investigators to produce a syndrome imitating hypophysis overfunction. This, I trust, I may convince you, has now been accomplished. Only the briefest abstracts of our work have been published, for we have regarded its immediate extension as important—its extension to hypophyseal ablations which Doctor Smith was ready to make and has energetically undertaken, and its extension to clinical cases, dependent upon further studies of anterior hypophyseal fluid with which a number of us have been concerned. Our efforts are not closed, indeed we would call them but begun, but results have already been secured which permit formulation and which permit us, I believe, to formulate the hypophysis problem with a somewhat different emphasis than that given it in the most recent reviews of the situation.

It is evident that to secure endocrine effects from dosage with any internally secreting tissue, we must have an appreciable storage of the specific secretion or hormone in the tissue cells or capillaries of the organ in question, and the hormone must be altered in the least possible way before conveyance to the test animal. Furthermore, while it is a basic conception that the internally secreting glands are constantly discharging small amounts of their products into the blood stream, no investigator

has adequately imitated this by sufficiently frequent dosage. These, at any rate, were the ideas which I felt must be rigidly adhered to in a final effort, begun in 1920, with the coöperation of Dr. Joseph A. Long, to secure characteristic effects from anterior hypophyseal substance.

It is proper to admit that we were "driven" to these standards by almost a year of fruitless medication with a great variety of commercial products, and with one product, TETHELIN, which our colleague Dr. T. B. Robertson had kindly placed at our disposal. Our research was primarily an attempt to show the relation of the various glands of internal secretion to the gonads and especially the ovary. And we were led into this field by the discovery of a remarkable clocklike four day ovulatory mechanism in the rat. The short ovulation cycle was brought out infallibly by the use of the method of vaginal smears which enabled Stockard and Papanicolaou for the first time to trace the ovulation cycle in the guinea pig. With so excellent a scheme for charting ovarian function in animals without harm to the animal, we felt that we had an unique criterion of detecting the relation of this function to the endocrine mechanism of the body. In addition to ablation experiments we sought to employ endocrine tissues or products. I shall not describe the details of our method here. Suffice it to say that a large amount of work and the most critical study which we could give showed that Stockard's beautiful study could be applied to the rat where rhythmic changes in the cell contents of the vaginal lumen were related just as definitely to œstrus and to ovulation, which follows œstrus by a definite number of hours. We tried many substances, thyroid, thymus, ovary itself, corpora lutea, adrenal cortex, adrenal medulla, and posterior and anterior pituitary. From only one of these tissues did we secure characteristic effects upon ovulation—from the anterior pituitary—effects which were also paralleled by still more striking changes in the body growth and in some of the internal organs besides the ovary, *e.g.*, the adrenal. To date we have carried out six series of experiments of this sort, totalling well over one hundred animals and

a hundred littermate sister controls. The first series was done from November 13, 1920 to May 20, 1921 and embraced seventy-six animals, a few of which were continued for a total period of a year. The second series was done with eighteen animals from June 8, 1921 to November 14, 1921. The third series from November 10, 1921 to January 25, 1922, and the fourth, fifth and sixth at six months intervals during the last year and a half.

Fresh bovine hypophyses have been secured from nearby slaughter houses, in all cases within a few hours of the death of the cattle, and split sagittally to permit rapid "shelling" out of the anterior lobe between thumb and scalpel, which is held accurately in the hypophyseal cleft—the vestigial lumen being well defined in cattle. It is, as you know, grown across in some forms, *e.g.*, in man, but in cattle one can separate pure anterior lobe tissue from the remainder of the gland which includes *pars nervosa* and *intermedia*, a singular nipple of anterior tissue beyond the cleft, and *pars tuberalis* on the stalk and brain floor (Fig. 3). This itself is very important from the standpoint of the possible specific physiology of the several components or lobes of the hypophysis. In this way only can one secure effects known to be due to the anterior lobe and only to this portion of the gland. There is no way to separate reliably the other components of the gland so as to secure with certainty only *intermedia* or *tuberalis* cells. Yet tissue which is chiefly *nervosa* and *intermedia* may be secured and these two components together were used as a "posterior lobe" substance in a series of control experiments which we conducted in addition to normal controls for the work with either portion of the gland. The hypophysis lobes were weighed in sterile dishes and thrown into 40 per cent. ethyl alcohol, agitated with glass rod for ten minutes so that every portion of the gland surface was thus washed, transferred to two changes of sterile normal saline and drained and thrown into a sterile mortar where they were triturated with force and speed for fifteen minutes with sterile glass or ocean sand. The exceedingly homogeneous paste was now diluted with saline in the proportion of one third the weight of the glands and the

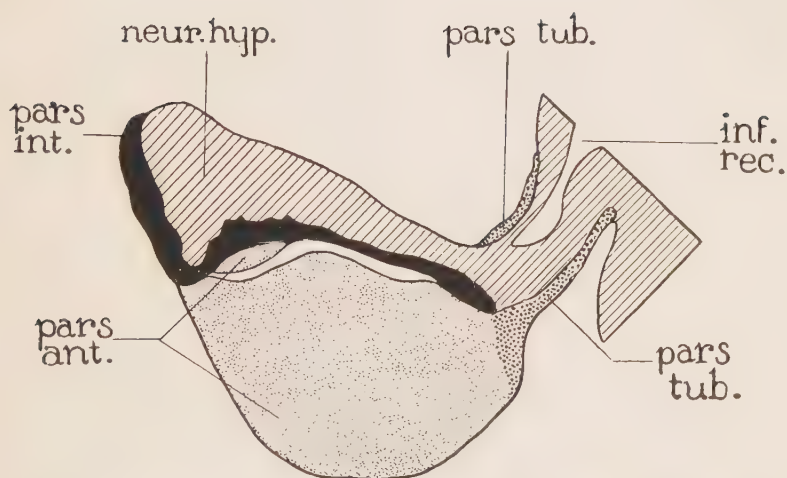


FIG. 3. —Schematic midsagittal section of the bovine hypophysis (from Biedl after Atwell and Marinus). The vestigial cleft (white) is seen between intermedia (black) and anterior (small dots) save where the nipple of anterior tissue occurs.

whole poured into large sterile centrifuge tubes and whirled at 3000 revolution for a half hour. The layers of sand, cell fragments and opaque pink fluid permitted the latter to be decanted. We planned to risk no possible adsorption of the hormone from filtration efforts and so injected the whole fluid with minimal delay intraperitoneally into a series of rats. In all cases litter-mate sisters were used as controls, and since we had the advantage of using litters with three or four sisters, another sister was given what I have described as combined nervosa and intermedia substance. Each cubic centimeter of the anterior fluid represented slightly over two grams of the beef anterior glandular substance, but since the anterior lobe as we separated it, crudely, weighs about 1.3 grams per animal, this was not quite the total material from two beeves. The posterior lobe cannot be administered in this concentration. The injections were begun with animals still suckling, on the 14th day of life (they were weaned on the 21st day) with injections of $\frac{1}{8}$ c.c. and twice doubled, so that eventually 1 or $1\frac{1}{2}$ c.c. were used. The vaginal smear method was used to detect the onset of sexual maturity and succession of ovulation cycles. The animals were selected so that if a discrepancy in weight resulted the lighter animals received anterior hypophyseal fluid. The anterior substance was a protein rich suspension difficult of ready absorption and had we submitted it to the subcutaneous tissues, absorption would have been slow and extensive local abscesses almost certainly on hand. The peritoneum could usually handle it, though in all instances the omentum betrayed evidence of extensive foreign body reaction-aseptic inflammatory responses. In some cases of massive dosage absorption was delayed so that the material heaped up and was merely walled off, and in one case, unfortunately infection occurred. *It is remarkable that almost from the beginning the anterior fluid animals grew better than did their controls*, either those untreated or those injected with the posterior portion of the gland, and this was true in the cases of extensive trouble in the peritoneum and even in septic infection far advanced, of which there was unfortunately one instance. Evidently here the

growth stimulus even overweighed the usual grave nutritional impairment which sickness from infection is known to bring, and which so quickly interferes with the growth curve of any animal not treated in the specific way. The growth superiority of the anterior hypophysis group became more marked in the third or post pubertal epoch of growth, for here, normally, as we know, adult stature is attained and a characteristic plateau of the growth curve results. The rat is a form which continues to grow slowly so that this epoch of growth is denoted rather by a sudden change in the growth curve and only very gradual ascent, rather than growth stasis. Due to the studies of Donaldson and his associates, and of many nutrition students we probably know more about the norm here than for any other animal form. An extensive material has been charted by him and we had in addition to the hypophysis controls the growth of several hundred animals used in many nutrition experiments of our own. *Rats treated parenterally with fresh anterior hypophyseal fluid invariably possess a greatly altered third, or post puberal growth curve.* The rate of growth does not at "adulthood" undergo the sudden decline characteristic for normal animals and indeed many of our rats did not sensibly alter their growth at this epoch. It was in this epoch consequently, in the epoch which begins somewhere between the ninetieth and one hundred and fiftieth day of life, that a very great disparity began to show in the growth of treated and untreated rats (Figs. 4, 5 and 6). The anterior rats, having to contend daily against the disadvantages of this treatment, were visibly becoming gigantic animals. In the first series of studies, six of these animals were treated for as long as nine months and two for four hundred days. Their controls were kept. Besides them, we had, as I have mentioned, the life growth charts of many rats.

The hypophysis giants were, in late stages, twice as heavy as the largest individuals known to us from our own and the published records for this animal species. Studies of these animals showed that they were not merely fat animals but that

FIG. 4.

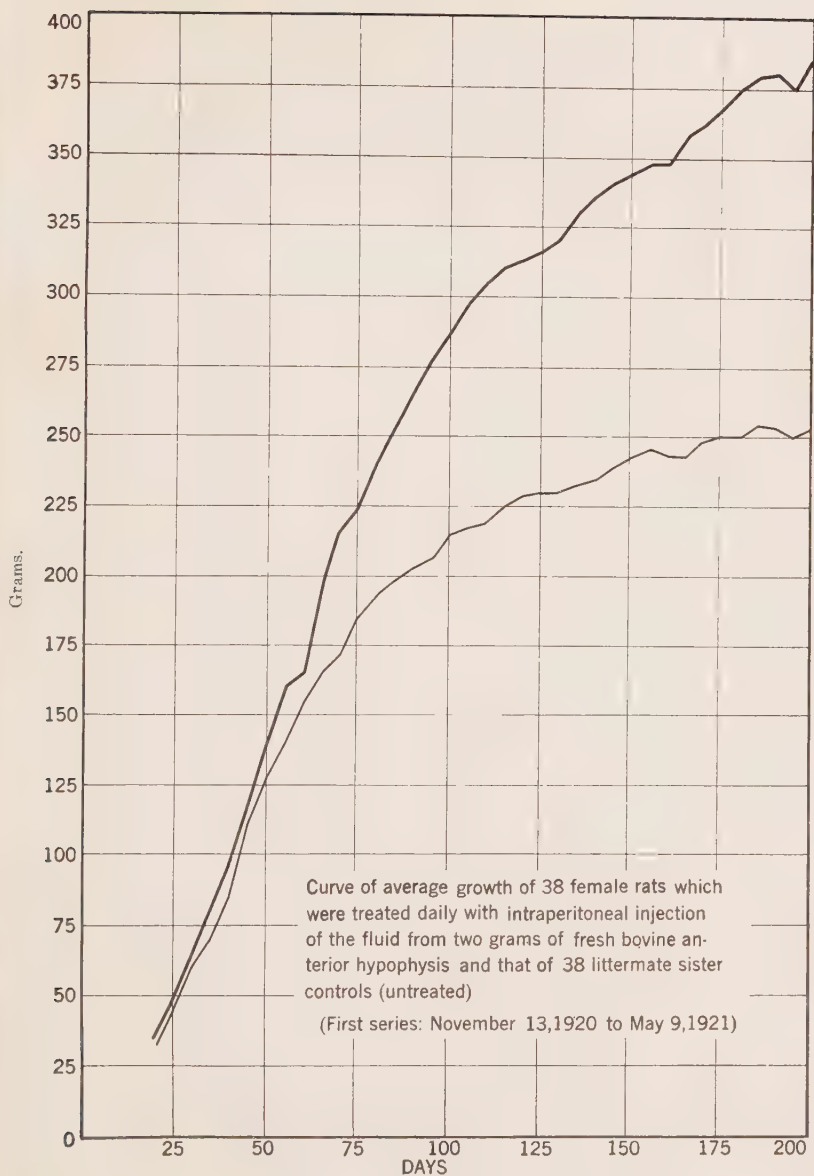


FIG. 5.

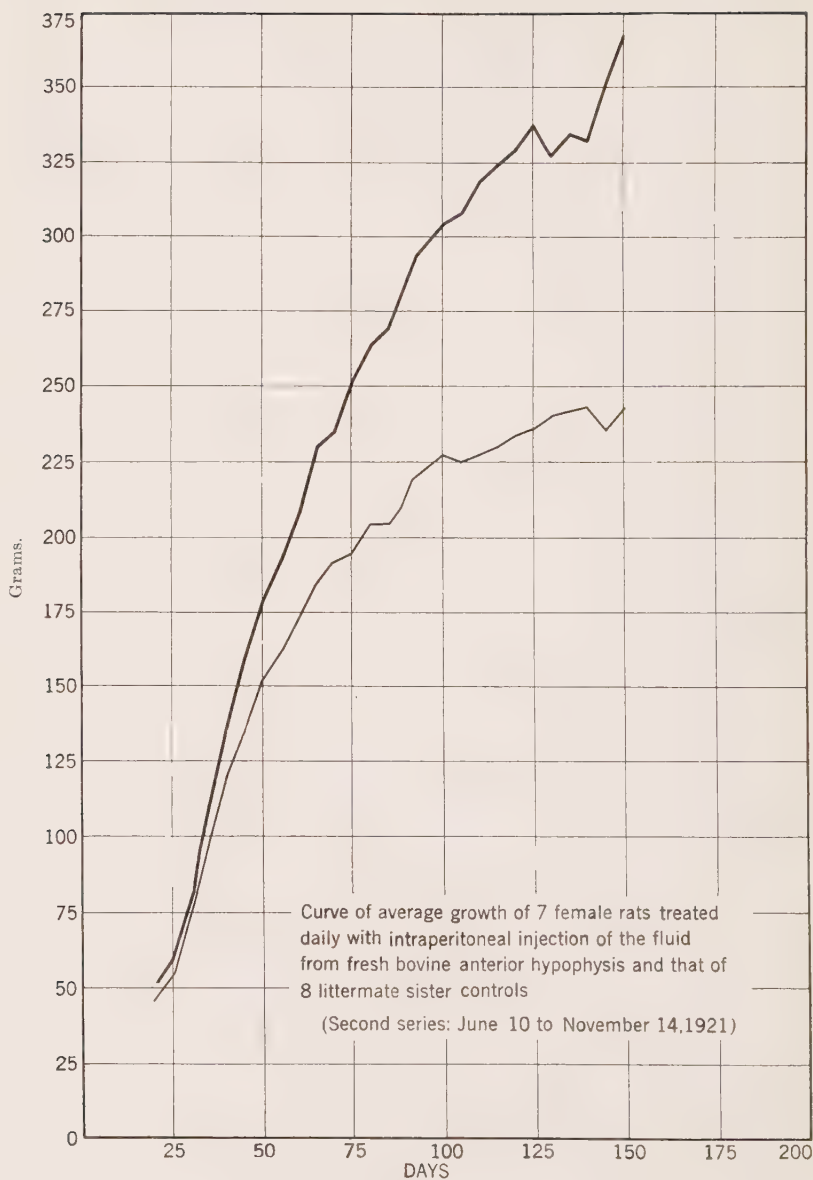
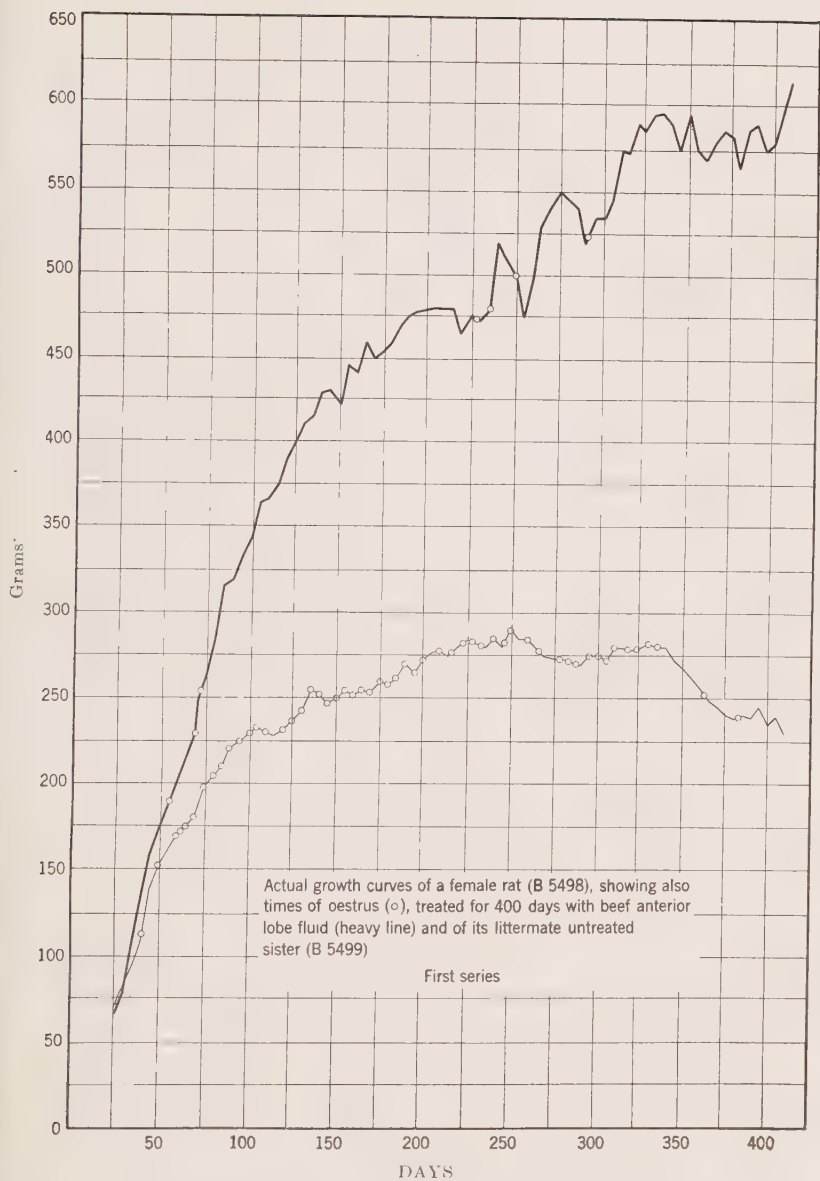


FIG. 6.



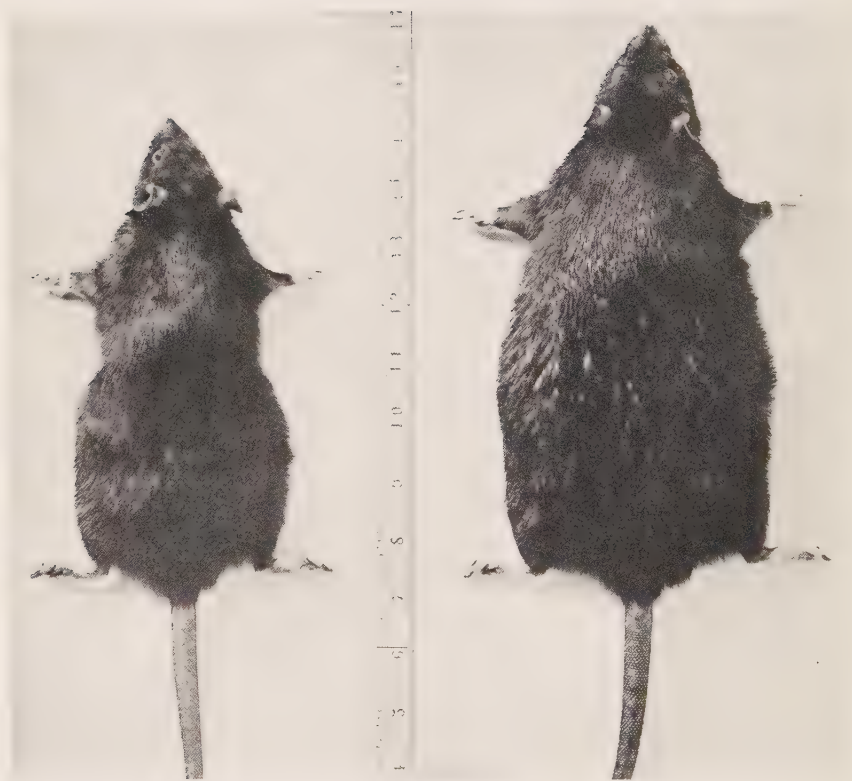


FIG. 7.—Photograph at time of autopsy (at somewhat over four hundred days of age) of two female rats whose growth curves and cycles are depicted in figure 6. Rat B 5498 received daily intraperitoneal injections of anterior lobe substance for over a year. Rat B 5499 is its littermate *untreated* control.

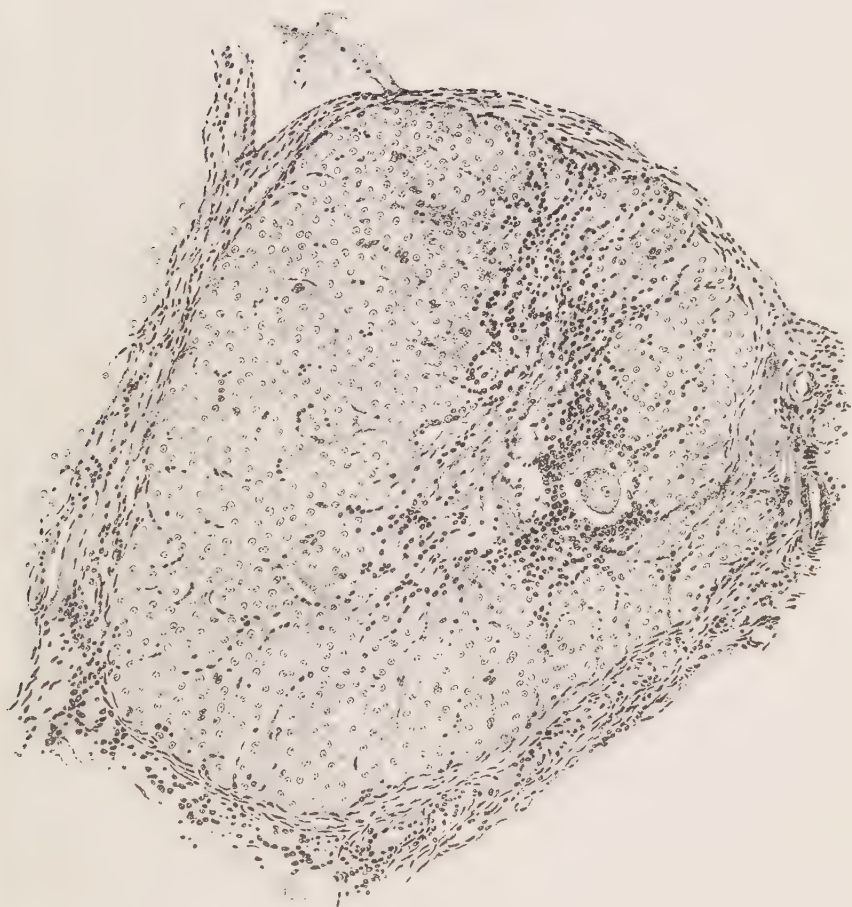


FIG. 8.—The production of corpora lutea in the walls of unruptured follicles—the typical effect of anterior hypophyseal treatment; the ovum remains in situ.

[illegible]



FIG. 9.—The partial production of lutein tissue in the walls of follicles; anterior hypophyseal treatment. (Rat 5533, 7-5-1).

a true overgrowth was participated in by the skeleton and by most of the viscera, especially the heart and lungs, liver, kidney and alimentary tract, but not by the reproductive tract, uterus and oviduct remaining infantile. (Fig. 7, Tables I and II).

Besides gigantism, another and equally characteristic effect is produced in the overbalance created by excessive anterior hypophyseal substance. The growth and maturation of ova is impaired or prevented, so that œstrus is infrequent or absent. (Tables III and IV). The newer studies in the physiology of reproduction have acquainted us with the exact time relations of œstrus and ovulation, and the necessary connection between the two, and furthermore, that the internal secretions of the ovary at this time bring about characteristic changes in the vaginal mucosa which thus announce, as it were, the ovarian cycle. Daily examination of the vaginal smear was made in all animals. It has previously been supposed that the conveyance of anterior hypophyseal substance might accelerate sexual maturity, on the basis of hyposexualism in Fröhlich's syndrome which was supposedly hypopituitarism. Goetsch thus believed he had hastened maturity by feeding the substance. Aside from the fact that our experiments of 1918 and 1919 with massive oral administration of anterior substance gave no effects in normal animals, a result confirmed by the studies of Frank and of Sisson and Broyles, it is interesting to note that just the reverse of this occurs when parenteral therapy is employed. The first œstrus usually occurred later in the treated animals and afterwards only a few ovulations occurred; in seven instances no single ovulation was observed. Sacrifice and necropsy of some of the hypophysis giants disclosed a characteristic picture in the ovary; ovulation had been prevented apparently through some toxic influence exercised on the ova, yet instead of follicular healing through death of the granulosa and hypertrophy of the thecal cells (the formation of so-called corpora atretica) the granulosa also had taken on growth changes and corpora lutea were produced enclosing the imprisoned ovum as you can see by these figures. (Figs. 8 and 9). These structures are normally produced only after follicular

rupture and their presence in the ovary is normally a wholly reliable sign of precedent ovulation and hence sexual maturity. But they were produced in the giants without ovulation and they can be produced even in the infantile gonads of suckling animals by administering anterior hypophyseal fluid. In the giants either the rate of formation of corpora or their survival exceeds that typical for normal ovaries for approximately twice as much lutean substance exists in these ovaries as in normal controls. It is evident then that in addition to germ cell injury, a specific stimulant to lutean cell growth occurs in these cases. The significance of this is not clear. One thinks of the hypophyseal hypertrophy of pregnancy and the corpora of pregnancy. Yet the ovum in pregnancy is undergoing an exuberant growth while the lutean tissue is rigidly confined to the follicles from whence issued the ova which were fertilized. It can be directly demonstrated that anterior fluid is not only toxic to ovarian ova but also to developing embryos. I mention these facts here especially for they are, as far as I know, the first evidence of direct gonadal injury in hypophyseal overfunction, a feature puzzling to many students of hypophyseal gigantism and acromegaly. Their general prevalence is indicated by the work of A. T. Walker with us, who has been able to sharply halt and indefinitely prevent ovulation in the hen.³ Now while these facts are true in cases of excessive anterior hypophyseal substance conveyed to the normal organism, it can be shown that lack of function of the sexual system may result from hypophyseal lack, and then the conveyance of the substance acts as a restorative of sex function. I shall refer to this later in describing substitution therapy in mammals after hypophysectomy.

The hyperpituitary giants may be characterized by another change obscure in nature but in harmony with our ideas of the relations obtaining between this gland and the adrenals, the cortex of which was seen to be reduced in amphibian anterior hypophysectomy. It is sometimes hypertrophied in the giants.

On the production of this train of endocrine effects from

³ It is surprising that the unquestionably less efficacious oral method of administration of anterior substance to birds has, in the hands of good observers, apparently acted as an ovulatory stimulant. (Clark, 1915.)

TABLE IV

Showing the Ovulation History of Thirty-eight Rats which Received Daily Intraperitoneal Injections of the Extract of the Fresh Anterior Lobe of the Beef Hypophysis Beginning with the Fourteenth Day of Life.

Designation of rat		Age in days at first œstrus	Length in days of subsequent cycles
G	5482	43	6-7-7 no more cycles
W	5483	94	9-19 no more cycles
W	5484	45	9-12-7-22 no more cycles
BH	5487	46	7-21 no more cycles
W	5403	45	7 no more cycles
B	5401	52	7-7-7-8-47-29-7-6-11-17-9 no more cycles
B	5490	53	6-7-8-22 no more cycles
W	5492	No cycles	
GH	5493	48	4 no more cycles
B	5498	65	7 no more cycles
BH	5497	45	14-7-13-8-7-7-7-5-6
W	5501	66	15 no more cycles
W	5500	64	9 no more cycles
B	5505	59	4-3-6-29 no more cycles
BH	5503	66	7-63-9 no more cycles
W	5509	65	12-3-40 no more cycles
BH	5511	57	4-9-9 no more cycles
BH	5510	57	6-7-8 no more cycles
BH	5514	61	11-4 no more cycles
GH	5513	49	5-7 no more cycles
W	5406	55	7-3-4-7-7-22-7 no more cycles
GH	5407	52	7-11-4-43 no more cycles
W	5517	88	14-12 no more cycles
W	5518	No cycles	
W	5409	65	No more cycles
B	5411	No cycles	
W	5521	57	7-5-31 no more cycles
W	5523	47	8-6-9-21 no more cycles
GH	5525	100	No more cycles
B	5529	50	7-14-28 no more cycles
W	5531	No cycles	
BH	5534	No cycles	
GH	5533	No cycles	
W	5542	46	7-15 no more cycles
B	5540	98	No more cycles
W	5537	58	11-11-4-6-7-7-7
GH	5541	No cycles	
GH	5545	47	36 no more cycles

parenteral dosage of mammals with anterior hypophyseal substance, it was clear that the time was ripe to test crucially whether or not all or even part of the syndrome of albinism in amphibian larvæ, especially the thyroid and interrenal subnormality were due to hypopituitarism. In spite of the ease with which the epithelial hypophysis of tadpoles had been separated from the embryonic brain, it was by no means certain that slight but significant brain injury had not been done; witness the situation in mammals. Moreover, even if, thanks to the pliability and regenerative power of embryonic tissues, actual nervous injury was avoided or restored, it was a fact that the posterior pituitary lobe fails to develop in all cases of successful anterior hypophysis ablation, contact of the epithelial and nervous components being necessary for this development. It was clear, then, that part of the syndrome of amphibian albinism might not be due directly to lack of the anterior pituitary lobe. B. M. Allen transplanted adult frog pituitary tissue into the albinous larvæ and secured very significant even if incomplete changes thereby, both a pigmentary darkening and beginning metamorphosis which he rightly interpreted as partly restorative in some way of thyroid normality. Swingle has confirmed these results. Now the hormones are not species specific and it took no persuasion to induce Smith to grasp the opportunity to make a therapeutic effort rather than transplantation to settle the question, for though oral administration had failed in all but growth, so had it failed to give any effects in normal mammals and yet remarkable ones had been secured by Evans, Long and me in the parenteral route. The use of specially made minute metal needles and syringe giving known dosages in graduations of only $1/600$ of a cubic centimeter, and with ice rather than narcosis for anesthesia, enabled the valuable albinous tadpoles to be given our anterior beef pituitary fluid. They darkened, grew normally, and metamorphosed in entirely normal fashion; as far as could be ascertained, there was a complete restoration from the effects of a removal of the anterior hypophysis. Especially interesting in this condition is the awakening, or activation and growth of the larval thyroid

which had been unable to develop with the hypophysis absent. The thyroid was now normal in size or hypertrophic. There was a similar restoration of interrenal substance. Here was a confirmation *ex-juvantibus* of the diagnosis of anterior hypophyseal responsibility for most if not the whole syndrome in hypophysectomized tadpoles.

Now though the security of the above conclusions seems assured, and though the three series of experiments I have mentioned establish firmly endocrine relations between hypophysis, thyroid, adrenal and sex glands, the recognition of a clinical disorder in the hypophyseal neighborhood (Fröhlich's syndrome) and the clever production of this syndrome (Cushing, Aschner, Biedl, Blair Bell, Smith), by experimental hypophysectomy in the mammal has brought to notice another symptom beside growth and sex trouble, a peculiar adiposity which when occurring is always linked with hyposexuality, hence the justifiable term, adiposo-genital syndrome. It has been natural to link this with the loss of or impairment of the hypophysis. Then came the remarkable and convincing work of Camus and Roussy of Paris, Baily and Bremer and others which gave this typical picture after sharply circumscribed and very slight hypothalamic injury, particularly of the superficial gray of the tuber cinereum. Besides other symptoms of lesions in the hypophyseal neighborhood, (polyuria and glycosuria) the adiposo-genital syndrome was repeatedly produced. Further, the literature now presents instances of the syndrome from suprasellar tumors of the brain neighborhood not involving the hypophysis; and finally, cases of the syndrome in defective cerebral development associated with other developmental defects. I have already mentioned Biedl's desire to hence recognize a pure cerebral and a pure hypophyseal Fröhlich, the latter, due not to brain involvement but to either injury of the pars intermedia (which he feels is alone concerned with the metabolic upset), or to pressure on its stalk and hence secretory channels into the brain. Although these secretory paths have never been convincingly shown, he thus explains the secondary production of Fröhlich's syndrome when an adeno-

matous tumor giving typical acromegaly grows so as to compress the stalk. It is difficult to see how he excludes pressure on the brain when we reflect what slight lesions or irritations affect the metabolic diencephalic centres, but it is evident that a predisposition to see a specific physiology in the intermedia with specific secretory channels is at the basis of this concept. He is equally positive about the Fröhlich from ablation of the gland as associated with no trace of brain injury, even the slightest. Since the brain lesions need be almost infinitesimal in the hands of a skilled operator, it seemed to us essential to test the theory by the production of the syndrome with as little brain injury as possible, and then to attempt to convey parenterally the hypophyseal hormones—at first those definitely known to be from the pars anterior, and so efficacious in the complete restoration of amphibia, and then also intermedia and nervous extracts, the latter especially since Cushing has reported an alteration of carbohydrate tolerance in hypophysectomized dogs on conveyance of these substances. It seemed important to do this in the rat since in this small and hardy mammal we could give a relatively massive dosage in replacement therapy—a dosage adequate even to “unbalance” a normal animal, as our early work had shown. Smith’s successful Fröhlich rats, produced by the injection of chromic acid into the gland, are probably the best examples secured of adipose distortion, for they may be best described as almost spherical in shape, veritable puff balls in appearance. To date perhaps twenty such pronounced types have been secured by him. When adiposity makes its appearance hyposexualism is coincident and anterior hypophyseal therapy is powerless to effect either metabolic or genital disturbance. On the other hand, the growth slowing or stasis regardless of whether it does or does not accompany the adipose syndrome is definitely repaired by the anterior hormone. More important has been the hypogenitalism and growth upset without adipose effects and the cure of such cases of sex infantilism by the anterior therapy which in some way is necessary for the testis and, in the female, provokes normal follicular growth and ovulation, for it is a proof of direct anterior hypophyseal responsibility for

gonadal undergrowth, quite independently of the recently proven (Camus & Roussy) astonishing mediation of the nervous system in the health of the reproductive glands, even though it would appear that both mechanisms must be intact as indeed must both pancreas and brain floor to give proper regulation to carbohydrate metabolism, (Claude Bernard's *piqûre*). We have hence the proof of the essentiality of hypophysis for body growth and for normal development of the sex gland. Unfortunately, it seems equally clear that anterior hormones cannot repair the distortion of metabolism called hypophyseal adiposity, and we are forced to conclude either that intermedia or nervosa therapy can clearly show the mediation of those portions of the gland, or failing that, hence show that nervous impairment alone is responsible for the peculiar obesity associated with any trouble near the basal diencephalon. Beside the failure as yet to secure curative effects from nervous and intermediate lobes, their participation is rendered less probable from the effects of radiation of adenomatous tumors beginning to establish the adipose syndrome. The treatment is undoubtedly toxic, as Bremer mentions, to all epithelial or glandular cells, and the resolution of a Fröhlich's syndrome by röntgenation makes it extremely probable that pressure and irritative effects of proliferating tissue on the brain constitute the essential cause of the Fröhlich metabolic upset. The failure of some complete hypophysectomies to establish the syndrome is thus explainable and their failure to cause sex disturbances in adults as reported by Houssay as due to the improbability of infantilism when body growth and differentiation have already taken place.

These then, comprise the chief facts for which I have claimed your attention. They do, I think, point to proven endocrine functions and relationships of the anterior hypophysis even if they seem to restrict greatly the cases of clear complicity of anterior underfunction and the therapeutic province of hormones of the anterior gland. The separation of hypophyseal infantilism and hypophyseal dwarfism is not yet at hand, yet evidence enough has been accumulated (Biedl, Erdheim, Simmonds, Priessl,

Peritz, Leri) to speak broadly of undergrowth types of hypophyseal cause—nansomia pituitaria—progeria perhaps being part of the special type. Ever since the studies of Rudolph Virchow, hypophyseal involvement in some types of cretinism has been known, and there are types which do not respond to thyroid any more than do most or many of the endemic type. I believe we have grounds for believing that the typical Brissaud's infantilism myxædematosis cured by thyroid has probably resulted in part from hypopituitarism secondary to thyroid lack, and it is by no means sure that we do not have primary hypophyseal deficiency in some other supposed thyroid defects not yielding to thyroid.

Before what may prove a most prolonged attack on the chemical nature of the anterior hormone or hormones, it is obvious that diagnostic or therapeutic use can be made of them in a range of conditions where the anterior gland may be involved. Yet to do this means creating a more readily absorbable and sterile fluid. These aims are in sight. When the proteins are coagulated by heat and alcohol and both the washed coagulum and fluid injected, these methods effectively destroy both the ovarian and growth effects. When alcohol is added so that its concentration never rises above 50 per cent., and the precipitated proteins removed by centrifugation, the fluid may give typical ovulation inhibitory effects, yet no growth stimulation. Indeed as low as 8 per cent. ethyl alcohol will kill the enzymes of autolysis and discourage bacterial activity so that the anterior fluid may be preserved a week without proteose formation and show both ovarian and growth effects. It would seem, then, that two hormones exist and that the growth hormone is lost at concentrations of alcohol between 8 per cent. and 50 per cent., or else that the ovulation inhibitory effect is secured by concentrations of the same hormone (in 50 per cent. alcohol) incapable of increasing growth. That the latter is not the case is indicated by the repeated production of gigantism with the whole fluid in animals where the normal ovulatory mechanism is preserved, an effect we have also recently secured with acid extracts. It is highly interesting in view of the existence of at least two types

of glandular or secretory cells in the anterior hypophysis that there seems to be also a topographical separation of growth and endocrine effects from anterior hypophysis suspension comparable with the topographical distribution of eosinophilic and basophilic cells. Smith has shown that the deeply pigmented stripe across the beef's anterior lobe (which may sometimes even be mistaken for the vestigial cleft) is attended with the accumulation of rather more basophils than elsewhere, the surface regions with relatively less basophils and many more eosinophilic cell types. Albino hypophysectomized frog larvæ demanding only very minute dosage constitute an ideal material for just such a test, and the center can be dug out of several bovine glands and the surface taken from others for tests; as a matter of fact the two sources gave in his hands respectively, chiefly the thyroid or metamorphosis hastening and the growth increasing effects.

Finally, acetic acid added as .2 Normal solution so that its final strength is .03 N (with a PH hence between 10^{-4} and 10^{-2}) precipitates most of the proteins and yet preserves both hormones. It is neutralized with NaOH. The fluid which is not quite clear of hæmoglobin is readily absorbed. Daily intraperitoneal treatment of animals for months gave a perfectly normal abdominal cavity without signs of irritation and no trace of residual material. The fluid lost its potency in both effects if passed through a Berkefeld, or submitted to pasteurizing heat. It seemed to us however probable that a hydrogen ion concentration could be found at which the proteins to which the hormones are presumably attached, would be in sufficiently different physical state to pass the filter, for the fluid is also stable to a considerable alkaline concentration. With Dr. Miriam E. Simpson a considerable series of studies was now directed towards the solution of this problem. We found that neutral alkaline solutions passed the Berkefeld. An excellent method for decreasing the protein moiety and yet preserving hormone effects has been found in alkaline extracts of the bowl mixture, which are later neutralized and the proteins insoluble at this juncture

removed by simple centrifugation. This fluid creates a remarkable gigantism.

Nothing is so necessary for scientific advance as the establishment of what shall be regarded as adequate criteria for our conceptions. Bailey's recent review of the physiology of the pituitary gland has been salutary, even though when he submitted to critical analysis the facts published prior to 1921 he concluded with what seemed to him necessary denial that any physiological rôle of the hypophysis had been placed beyond doubt. With complete acceptance of the newer physiology of the 'tween brain, we may nevertheless be certain:

Firstly, that the anterior hypophysis is indispensable for growth to adult stature, a lessened amount of its hormone being the direct cause of an important group of, if not all, endocrine dystrophies, and an increased amount of the hormone the direct cause of overgrowth.

Secondly, the hypophysis stands in a necessary relationship to normal function of the thyroid, sex glands and adrenal cortical tissue. Any explanation of how these effects are mediated constitutes part of the ill understood field of chemical dependencies and correlation within the body, ignorance of which cannot properly place in doubt evidence that that correlation exists.

BIBLIOGRAPHY

- Allen, B. M.: The result of extirpation of the anterior lobe of the hypophysis and the thyroid of *Rana pipiens* larvæ. Science, N. Y. & Lancaster, Pa., 1916, xliv, 755-757.
- Extirpation of the hypophysis and thyroid glands of *Rana pipiens*. Anat. Record, Phila., 1916-17, xi, 486.
- Effects of the extirpation of the anterior lobe of the hypophysis of *Rana pipiens*. Biol. Bull., Woods Hole, Mass., 1917, xxxii, 117-130.
- Aschner, B.: Ueber die Beziehungen zwischen Hypophysis und Genitale. Arch. f. Gynaek. Berl., 1912, xcvii, 200-228, 1 pl.
- Ueber die funktion der Hypophyse. Arch. f. ges. Physiol., Bonn, 1912, 1-146, 1 pl. Quoted in von Fürth's cxlvi.
- Hypophyse und Diabetes insipidus. München med. Wehnschr., 1917, lxiv, 422.

- Cytological observations on the pars buccalis of the hypophysis cerebri of man, normal and pathological. *J. Med. Research, Bost.*, 1920-21, xlii, 349-381, 1 pl.
- Bailey, P.: Die funktion der Hypophysis cerebri. *Ergebn. d. Physiol.*, 1922, xx, 162-206.
- and Bremer, F.: Experimental diabetes insipidus. *Arch. Int. Med.*, Chicago, 1921, xxviii, 773-803.
- Experimental diabetes insipidus and genital atrophy. *Endocrinology*, Glendale, Calif., 1921, v, 761.
- Bell, W. B.: Experimental operations on the pituitary. *Quart. J. Exper. physiol.*, Lond., 1917, xi, 77-126.
- The pituitary body and the therapeutic value of the infundibular extract in shock, uterine atony and intestinal paresis. *Brit. Med. J.*, Lond., 1909, ii, 1609-1613.
- The pituitary: A study of the morphology, physiology, pathology and surgical treatment of the pituitary, together with an account of the therapeutical uses of the extracts made from this organ. Lond., 1919, Builliery, Tindall & Cox, 348 pp., 7 pl.; also N. Y., 1919, Wood, 368 pp., 8°.
- Biedl, A.: Referat über die Hypophyse. *Verhandl. d. deutsch. Gesellsch. f. innere Med.*, München, 1922, xxxiv, Kong., 331-334.
- Ueber die Hypophysis. *München med. Wchnschr.*, 1922, lxix, 796.
- Physiologie und Pathologie der hypophyse. Referat gehalten am XXXIV Kongress für innere Medizin in Wiesbaden, 26 April, 1922. München, 1922, Bergmann, 81 pp., 8°.
- Innere Sekretion—Ihre physiologischen Grundlagen und Ihre Bedeutung für die Pathologie. Berlin u. Wien. 1916-1922, Urban & Schwarzenberg, 3 vols., 672 pp., 939 pp., 480 pp., 8°, vols. i and ii, 3rd edition, vol. iii, 4th edition.
- Bremer, F.: Considerations sur la pathogénie du diabète insipide et du syndrome adipo-génital. *Rev. neurol.*, Par., 1922, xxix, 622-639, 644-648.
- Brissaud: L'infantilisme vrai. *I Congr. de la Salpêtrière*, 1907, xx.
- Camus, J. and Roussy, G.: Experimental researches on the pituitary, diabetes insipidus, glycosuria and those dystrophies considered as hypophyseal in origin. *Endocrinology*, Glendale, Calif., 1920, iv, 507-522.
- Syndrome adipo-génital et diabète insipide expérimental (présentation d'un chien). *Compt. rend. Soc. de Biol.*, Par., 1921, lxxxv, 296.
- Les fonctions attribuées à l'hypophyse. *J. de physiol. et de path. gén.*, 1922, xx, 509-535.
- and LeGrand, A.: Un cas de diabète insipide par lésion de l'infundibulum. *Compt. rend. Soc. de Biol.*, Par., 1922, lxxxvi, 719-722.
- Les syndromes hypophysaires anatomie et physiologie pathologiques. *Rev. neurol.*, Par., 1922, xxix, 622-639.

- Hypophysectomie chez le chien et le chat. Technique et resultats de 149 interventions. *Compt. rend. Soc. de Biol., Par.*, 1922, lxxvi, 1008-1010. L
- Cannon, W. B.: Conditions affecting secretion of the thyroid gland. *Tr. Ass. Am. Physicians, Phila.*, 1916, xxxi, 162-164.
- Clark, L. N.: The effect of pituitary substance on the egg production of the domestic fowl. *J. Biol. Chem., Baltimore*, 1915, xxii, 485.
- Crooke, H.: *Micrographia*. Lond., 1615, W. Jaggard, 1111 pp., 10 pl., fol.
- Cushing, H.: The pituitary body and its disorders; Clinical states produced by disorders of the hypophysis cerebri. *Phila. & Lond.*, 1912, J. B. Lippincott Co., 351 pp., front, roy., 8°.
- Degener, L. M. and Livingstone, A. E.: The effect of thyroidectomy and castration respectively on the weight of the pituitary in the rabbit. *Am. J. Physiol., Baltimore*, 1913, xxxi, 24.
- The effect of thyroid extirpation on the hypophysis cerebri in the rabbit. *Quart. J. Exper. Physiol., Lond.*, 1913, vi, 111-118.
- Donaldson, H. H.: The Rat. *Phila.*, 1915, Wistar Inst. Ant. & Biol., 278 pp., 8°, *Memoirs. Wistar Inst.*, No. 6.
- Erdheim, J.: Nanosomia pituitaria. *Zeiglers Beitr.*, 1916, lxii, 302.
- Evans, H. M., and Long, J. A.: The effect of feeding anterior lobe of the hypophysis on the œstrous cycle of the rat. *Anat. Record, Phila.*, 1921, xxi, 62.
- The effect of anterior lobe administered intraperitoneally upon growth and maturity and the œstrous cycles of the rat. *Anat. Record, Phila.*, 1921, xxi, 62.
- Characteristic effect upon growth, œstrus and ovulation induced by the intraperitoneal administration of fresh anterior hypophyseal substance. *Proc. Nat. Acad. Sc., Balt.*, 1922, viii, 38.
- Flower, C. F., Forkner, C. E., Kellum, W. E., Walker, A. T., Smith, P. E., and Evans, H. M.: Stability of hormones in the anterior hypophysis. *Anat. Record, Phila.*, 1923, xxv, 107.
- et al.* Separation of the Principle in the anterior hypophysis affecting ovulation from that controlling general body growth. *Anat. Record, Phila.*, 1923, xxv, 107.
- Frank, R. T.: Influence of pituitary extracts on the genital tract. *J. Am. M. Assn., Chicago*, 1919, lxxiii, 1764-1765.
- Fröhlich, A.: Ein Fall von Tumor der Hypophysis cerebri ohne Akromegalie. *Wien. klin. Rundschau*, 1901, xv, 883-906.
- Die Pharmakologie der Hypophysensubstanzen. *Wien. med. Wehnschr.*, 1914, lxiv, 1061-1065.
- Goetsch, E., and Cushing, H.: The pars anterior and its relation to the reproduc. glands. *Proc. Soc. Exp. Biol. & Med., N. Y.*, 1913, xi, 26-27.
- Goetsch, E.: The influence of pituitary feeding upon growth and sexual development. An experimental study. *Johns Hopkins Hosp. Bull., Baltimore*, 1916, xxvii, 29-50.

- The relations of the pituitary gland to the female generative organs; from the experimental and clinical aspects. *Surg. Gynec. & Obst.*, Chicago, 1917, xxv, 229-243.
- The relation of the pituitary gland to the female generative organs. *Tr. Am. Gynec. Soc., Phila.*, 1917, xlii, 11-40, Discussion: 310-326.
- Gudernatsch, J. F.: Fütterungsversuche an Amphibienlarven. *Zentralbl. f. Physiol., Leipz. u. Wien*, 1912, xxvi, 323-325.
- Feeding experiments on tadpoles. II. A further contribution to the knowledge of organs of internal secretion. *Amer. J. Anat., Phila.*, 1915, xv, 431-482.
- Hogben, L. T. and Winton, F. R.: The pigmentary effector system, III Color response in the hypophysectomised frog. *Proc. Roy. Soc. Lond.*, 1923-24, S.B., xcv, 15-31, 1 pl.
- Houssay, B. A.: Les contradictions dans les études sur les actions des extraits hypophysaires. *Compt. rend. Soc. de Biol., Par.*, 1921, lxxxv, 33.
- Leri, A.: Chapter on Acromegaly in Lewandowsky's "Handbuch der Neurologie," Berl., 1910, iv, 283-336.
- Levi, E.: Contribution à l'étude de infantilisme du type Lorin. *N. inconog. de la Salpetriere, Par.*, 1908, xxi, 297, 5 pl., 421.
- Léopold-Lévi: Diagnostic des petits syndromes hypophysaires. *Médecine, Par.*, 1922-23, iv, 342-348.
- Hyperhypophysie paroxystique et réactionnelle. *Rev. neurol., Par.*, 1922, xxix, 705-709.
- Long, J. A., and Evans, H. M.: The œstrous cycle in the rat and its associated phenomena. Berkley, Calif., 1922, Univ. of Calif. Presse, 148 pp., 11 pl., 4°. *Memoirs, Univ. of Calif.*, vol. 6.
- Marie, P.: Sur deux cas d'acromégalie, Hypertrophie singulière non congenitale des extrémités supérieures, inférieures et cephalique. *Rev. de Med., Par.*, 1886, vi, 297-333.
- De l'ostéo-arthropathie hyperthrophique pneumique. *Rev. de Med., Par.*, 1890, x, 1-36.
- Acromegaly. *Brain, Lond.*, 1890, xii, 59-81.
- Peritz, G.: Article "Akromegalie und Gigantismus" in Kraus u. Brugsch. "Spezielle Pathologie und Therapie innerer Krankheiten," Berl. u. Wien., 1919, Urban & Schwarzenberg, i, 627-679.
- Kinder mit hypophysärer Adipositas. *München. med. Wchnschr.*, 1920, lxvii, 141.
- Priessl, A.: Ueber hypophysären Zwergwuchs. *München. med. Wchnschr.*, 1920, lxvii, 59.
- Rathke, H.: Ueber die Entstehung der glandula pituitaria. *Arch. f. Anat., Physiol. und wiss. Med.*, 1838, 482-485.
- Robertson, T. B.: Experimental studies on growth. IV. The influence of tethelin, the growth-controlling principle of the anterior lobe of the pituitary, upon the growth of the white mouse. *J. Biol. Chem.*, Baltimore, 1916, xxiv, 397-408.

- Recent investigations on the influence of the anterior lobe of the pituitary body upon growth and on the properties of the growth controlling constituent, tethelin. *Endocrin.*, Los Angeles, 1917, i, 24-35.
- Rogowitsch, N.: Die Veränderungen der Hypophyse nach Entfernung der Schilddrüse. *Beitr. a. path. Anat. u. z. allg. Path.*, Jena, 1888-89, iv, 453-470, 1 pl.
- Simmonds, M.: Atrophie des Hypophysenvorderlappens und hypophysäre Kachexie. *Deutsch. med. Wchnschr.*, Leipz. u. Berl., 1918, xlv, 852-854; also *München. med. Wchnschr.*, 1918, lxxv, 441.
- Zwergwuchs bei Atrophie des Hypophysenvorderlappens. *München. med. Wchnschr.*, 1919, xlv, 486.
- Sisson, W. R., and Broyles, E. N.: The influence of the anterior lobe of the hypophysis upon the development of the albino rat. *Bull. Johns Hopkins Hosp.*, Baltimore, 1921, xxxii, 22-30, 2 pl.
- Smith, P. E.: Experimental ablation of the hypophysis in the frog embryo. *Science*, Lancaster, Pa., 1916, n. s., xlix, 280-282.
- The growth of normal and hypophysectomized tadpoles as influenced by endocrine diets. *Berkeley, Calif.*, 1918, Univ. of Calif. Press, 22 pp., 8°.
- The pigmentary, growth and endocrine disturbances induced in the anuran tadpole by the early ablation of the pars buccalis of the hypophysis. *Phila.*, 1920, Wistar Inst. Anat. & Biol., 112 pp., 8°, *Amer. Anat. Memoirs*, No. 11.
- and Smith, I. P.: The effect of intraperitoneal injection of fresh anterior lobe substance in hypophysectomized tadpoles. *Anat. Record*, *Phila.*, 1922, xxiii, 38.
- and Walker, A. T., and Graeser, J. B.: The production of the adiposogenital syndrome in the rat, with preliminary notes upon the effects of a replacement therapy. *Proc. Soc. Exper. Biol. & Med.*, N. Y., 1923, xxi, 204-206.
- Stockard, C. R., and Papanicolaou, G. N.: The existence of a typical oestrous cycle in the guinea pig; with a study of its histological and physiological changes. *Am. J. Anat.*, *Phila.*, 1917, xxii, 225-283, 9 pl.
- Swingle, W. W.: Iodine as the active principles of the thyroid gland. *Endocrinology*, Los Angeles, Calif., 1918, ii, 283-288.
- Virchow, R.: Die krankhaften Geschwülste. Berlin, 1863. Fötale Rachitis, Kretinismus und Zwergwuchs. *Virchow's Arch.*, 1883, xciv.
- Walker, A. T.: The effect of injections of anterior lobe substance of pituitary upon ovulation in the chicken. Thesis, M. A., Univ. of Calif., 1923, Unpublished.
- Wulzen, R.: The morphology and histology of a certain structure connected with the pars intermedia of the pituitary body of the ox. *Anat. Record*, *Phila.*, 1914, viii, 403.
- Zondek, H.: Die Krankheiten der Endokrinen Drüsen. Berlin, 1923, Springer, i-vii, 316 pp.

RENAL SECRETION AND RENAL DISEASES¹

DR. LUDWIG ASCHOFF

Professor of Pathology and Anatomy, University of Freiburg
1/Breisgau, Germany

WHEN last in New York, in 1913, I devoted one of the Cartwright lectures to an analysis of my views on the structure of the kidney and the various forms of nephrosclerosis. Some of you may remember the lecture and it may perhaps be a matter of surprise to you that I should choose the same subject today. But in the interim so many new facts concerning the physiology of the kidney have been revealed, that it becomes necessary to reconcile our knowledge of the morphology of the organ with these newer views.

First of all, the book by Cushny must be mentioned, which again restores some confidence in the old filtration theory of Ludwig. According to this theory, the glomeruli play the chief rôle, the total urine being formed there by a simple filtration process. Then in passing through the contiguous convoluted tubule, there occurs a certain amount of resorption of those substances that are needed in order to maintain their normal threshold level in the blood—for example, sodium chloride. There is little or no resorption of substances which are not needed to maintain such a threshold level, nor of substances which are normally foreign to the body. This filtration theory necessitates a renewed study of renal morphology and of its relation to renal function.

As is, perhaps, well known, vital staining has proved itself a splendid aid for the study of renal secretion. In 1913, I was able to make a detailed report concerning the excretion of carmine through the kidney, based upon Suzuki's studies. If I may be permitted to digress for a moment, I should like to repeat

¹ Delivered May 17, 1924.



FIG. 1.—Intravital staining of the convoluted tubules of the kidney with lithium carmine. Advanced stage of excretion. The various parts of the tubules are stained differently. (After Suzuki.)

the salient facts revealed by this investigation because they represent the basis for subsequent histologic investigations.

Since the experiments of Heidenhain, the long-known fact that the epithelial cells of the kidney can be stained with the help of dyes has been interpreted repeatedly, in the sense that the staining of the epithelial cells, or more correctly, the formation of colored granules within the cells, is the visible expression of a secretion. The dye is said to be excreted from the renal epithelium in the form of colored granules. The peculiar fact that the different urinary tubules are stained with varying intensity was thought to mean that the different tubules *function* with varying intensity—a part working while another part rests. The investigations of Suzuki have shown that the formation of pigment granules within the cells has nothing whatever to do with the secretion of the dyestuff itself, and is rather a purely accidental accompaniment, a sort of storage process that catches up a part of the dyestuff passing through the epithelium. It is only a sort of indicator of the passage of the dyestuff through the cells, and does not itself represent the process of excretion. According to Suzuki, the unequal staining of the tubules does not depend on their varying activity, but rather on the fact that the primary convoluted tubules store the dye to a great extent, and indeed mostly, in the proximal portion close to the point of departure from the glomerulus, and least in the distal portion at the transition of the loop of Henle. The primary convoluted tubule can therefore be divided into four sections which gradually merge with one another and which were designated by Suzuki as the proximal, intermediary, distal and transitory portions. Finally, Suzuki was able to determine that the earliest evidence of secretion consists in a very fine granular precipitation of the dyestuff on the brush border of the epithelial cells, and that the granular staining of the cells themselves only occurs later.

These investigations have since been confirmed by others, but the question has not as yet been conclusively answered concerning the exact mechanism responsible for the remarkable storage of vital dyes within the renal epithelium. Suzuki and I felt that

the dyestuff first of all passed through the glomeruli in the manner of a filtration, but that simultaneously there also occurred a very much feebler passage of dye through the epithelium of the primary convoluted tubule in the manner of a secretion. We interpreted the above-mentioned fine granular precipitate in the brush-like marginal membrane of the renal epithelium as the earliest evidence of this secretory process. At this early period, the epithelium itself was still entirely free of carmine granules. We believed that the granular deposit of dye within the cell bodies developed only very gradually in the course of the prolonged passage of dyestuff through the epithelium. Even at that time however, we were obliged to admit the possibility that the reverse route, a resorption of dyestuff from the lumen, might conceivably lead to the same intracellular pictures. We were unable to solve the problem solely with the aid of vital staining, and at that time expressed ourselves more in favor of a secretion theory. For this reason, we named the primary convoluted tubule, the secretory portion of the renal tubular labyrinth.

On the other hand, the occurrence of the carmine-colored casts in the Henle loops impelled us to assume that resorption of fluid took place at this site. Peter claims to have observed that those animals whose kidneys possess conspicuously long Henle loops, excrete a markedly concentrated urine. This served to confirm our belief that a concentration of the urine took place within the Henle loops. For this reason the narrow limit of the Henle loops was designated the resorptive portion of the renal tubular labyrinth.

This represented the general status of the subject at the time when the recent work at the Pathological Institute of Freiburg was begun two years ago by Professor Mitamura. Suzuki having conclusively demonstrated that the appearance of vitally staining intracellular granules did not coincide with the curve of renal excretion, it now became desirable to ascertain whether the intracellular deposit of dyestuff within the epithelium of the primary convoluted tubules was the result of a secretory or of a resorptive process. The mooted question as to whether the

filtration or the secretion theory was the correct one was therefore subjected to re-analysis from the standpoint of morphology.

The investigations received their first stimulus from the observations of Moellendorff, who confirmed the essential findings of Suzuki, but demonstrated that the deposits of dye within the epithelium of the primary convoluted tubules did not coincide with the vital granules, the chondrikones and chondriosomes; and in fact that the degree and type of the deposits were entirely independent of the chondriosome structure of the various cells. As a result of the work of Doctor Oka, I myself had pointed out that the mitochondria and the vitally stained granules respond to autolysis quite differently. We could very easily distinguish that the mitochondria, the so-called Altmann granules, very rapidly succumb to cadaveric autolysis, whereas the carmine-colored granules resist autolysis for a remarkably long time. Also, the arrangement of the mitochondria and of the carmine granules, although they superficially seemed to show some structural resemblances to one another, present important differential characteristics. The carmine granules are densest in arrangement in the neighborhood of the nucleus, and do not extend as far into the basal portion of the cells as do the mitochondria. This doubt concerning the identity of mitochondria and of the vitally stained granules has been referred to in the work of Schulemann, Evans, Moellendorff and others. Of these observers, Moellendorff was able to demonstrate that granular vital staining is the result of a condensation process which takes place quite independently of the mitochondria, and in fact, occurs outside of or between them. These observations can be accepted as at least applying to the *acid* dyes. Moellendorff even pointed out that the carmine granules seem to move their position from the marginal membrane downward toward the base, an observation which would certainly speak in favor of a resorptive process.

In Cushny's publication, which appeared in 1917, a careful critical analysis was made of all the contradictory observations which had appeared in the literature. He attempted to reestablish Ludwig's filtration theory in a modified form, and has

again actively renewed the discussion of secretion versus resorption. Since the publication of Cushny's book, quite a confusion of viewpoints has again appeared.

In the light of the present knowledge concerning the excretory mechanism of the kidney it becomes increasingly important to attempt to solve this problem of renal function by means of all possible methods, physicochemical, physico pharmacological and pathological. I have, therefore, taken up the subject again in coöperation with Professor Mitamura from a pure morphological standpoint. In choosing a method of study, we decided upon what is perhaps the oldest one, the investigation by means of dyes, and confined ourselves exclusively to the use of lithium carmine. This substance possesses many advantages, such as permanency of its solutions, simplicity of composition, the possibility of colorometric titrability, and the absence of a leuco (colorless) base.

When I was in Philadelphia a short time ago, Professor Richards told me of his most noteworthy attempt at the chemical investigation of the contents of the glomerular capsule on the one hand, and the urine on the other. These investigations bring an unequivocal proof of the correctness of the modified filtration theory. My elucidation might, therefore, appear superfluous, were it not a welcome addendum from a purely morphological standpoint. I feel myself the more justified to do so as a paper of Stieglitz in one of your leading journals points to an opposite opinion.

These physiological investigations upon the normal carmine excretion in rabbits demonstrated that, just as with phenolsulphothalein, carmine was excreted in very much larger amounts and greater concentration in the first few hours than subsequently. The greater the concentration of carmine in the blood, the greater was the excretion of carmine in the urine. On the other hand, the concentration of carmine in the urine may be more than ten times the concentration in the blood. Furthermore, after the first few hours, the excretion of carmine in the urine is uninfluenced by the volume of urine. This is evidence

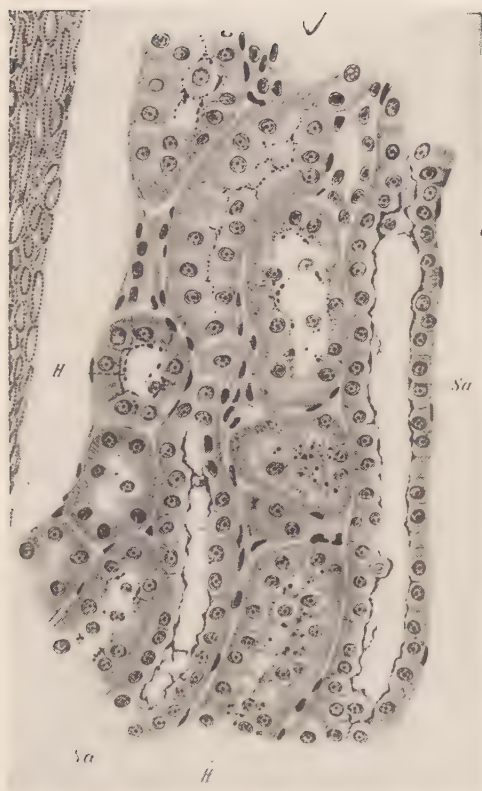


FIG. 2.—Earliest evidence of excretion of lithium carmine in the kidney. Finely granular deposits in the brush border of the epithelial cells. (After Suzuki.)

that carmine belongs to the group of substances which Cushny classifies as belonging to the group possessing no threshold value.

From these observations, Mitamura concluded that the excretion of dyes occurs with greatest constancy within the first few hours following their administration. Mitamura could also confirm Suzuki's observation that the first evidence of dyestuff excretion in the kidney, as far as it can be morphologically recognized, consists in a finely granular precipitate deposited along the brush-like marginal membrane of the epithelium of the primary convoluted tubules. The injection of larger doses, as well as the reduction in the volume of urine, results in increased precipitation at this site. This precipitate, which one would judge from its color, consists of pure masses of carmine, is deposited only in the primary convoluted tubules, never in the loops.

Another important finding concerned the formation of the intracellular granules of dye. The very first traces appeared relatively early, within fifteen minutes after an injection. At this early stage they appear as exceedingly fine deposits which can be recognized only with difficulty, and which lie immediately beneath the marginal membrane of the cells. In the course of the next few hours these granules increase in size, in number and in intensity of their color. They then appear very definitely to be migrating gradually deeper in toward the base of the cell. After twelve to twenty-four hours, in other words, at the height of this granular, intracellular deposition, they are situated for the most part at about the level of the mid-line of the nucleus, or even deeper in toward the base of the cell. During this period, when the carmine granules are moving toward the base, they begin to arrange themselves in rows so as to give the appearance of the rod-like arrangement of the mitochondria. For this reason one can so easily confuse them with mitochondria which have undergone granular degeneration, although as a matter of fact they really lie in between the mitochondria. Later, that is to say, after forty-eight hours, there occurs a clumping together of granules of dye, such as Suzuki described in detail. The agglu-

tinated masses eventually come to lie within vacuoles of the cell, as evidence of regression of the vital storage phenomenon.

During the entire period of excretion of the dye there never occurs, not even in the early stages of excretion, any diffuse coloration of the epithelium of the primary tubules, such as one might expect to see during secretion; so that one must conclude that only an extremely dilute concentration of the dye could have permeated the cell. This observation agrees with the conception expressed by Suzuki, that the dye is for the most part excreted through the glomeruli and only a small portion passes through the tubules. The above-described morphological pictures, such as the appearance of granular precipitate in the marginal membrane, the appearance of dye in dust-like granules beneath the marginal membrane and the gradual migration of these granules toward the base of the cell, all indicate that the dye is absorbed in extreme dilution from the lumen of the tubules. It passes within the cell in a contrary direction to the blood stream, comes to lie between the mitochondria, and eventually condenses into coarsely granular shapes.

The question now arises, what rôle do the glomeruli play in normal animals stained with carmine? In spite of the fact that he employed as fixation a lead acetate solution, Mitamura was never able to find any carmine deposits within Bowman's capsule. In other words, the dye must be excreted here in extreme dilution.

One is very much tempted to assume from these pictures that carmine is excreted by the glomeruli in extremely dilute form, and that a concentration takes place within the primary convoluted tubules as a result of the resorption of water and of certain salts. However, other evidence is needed to support this view. For this reason, Mitamura performed two other groups of experiments, in one of which he experimentally induced a glomerular damage, in the other a purely tubular destruction. In the first group of experiments he employed Habu-toxin, the poison of a Japanese snake, which damages the glomerular capillaries, similar to the injury which according to Pearce, Flexner and Noguchi is induced by the poison of the American rattle-snake.

For the purpose of producing a tubular damage, he employed chromium and bichloride of mercury. A remarkable fact was revealed by these experiments, namely, that the interference with carmine excretion was quite similar in both groups. Both series of experiments presented all gradations, from the mildest to the severest forms of disturbance, in the staining of the tubules. It was therefore necessary to assume in both instances the disturbance in carmine excretion was dependent upon a common factor. Whenever the carmine excretion was entirely suppressed, by damage of the glomeruli, no vital staining of the epithelium of the primary convoluted took place, in spite of the fact that the mitochondria were, as a rule, well preserved, at least in the proximal portions of the primary tubules. There was, therefore, no obvious reason why these epithelial cells could not excrete carmine and take the vital stain. One must rather assume that the carmine never reached them because of the fact that it was not excreted by the glomeruli.

On the other hand, in some of the animals whose tubules had been destroyed, and in whom a marked polyuria was produced by means of large doses of sodium chloride, a very good excretion of carmine took place, and yet the epithelium showed absolutely no vital staining. In these animals 40 c.c. of urine was excreted in one hour, about ten times the normal volume. If only a small part of this volume had passed through the epithelium, one would have expected them to have taken the vital stain. But here the resorption through the epithelium had been interfered with by the concentration of sodium chloride and for this reason no vital staining occurred. We are therefore obliged to assume that when there exists a severe injury, either to the glomeruli or to the tubules, the resultant disturbance in dye excretion must always be ascribed to a disturbance in the function of the glomeruli and not of the epithelium.

The third group of Mitamura's investigations concerned the result of section of the spinal cord. In rabbits on whom such an operative procedure had been performed, all possible diuretic influences were excluded by a period of observation varying from

seventeen minutes to one hour. When the blood pressure of these animals fell to 30 mm. of mercury (100 mm. being the normal), no carmine precipitate could be observed in the primary convoluted tubules. Vital staining of the epithelial cells also failed to occur.

On the other hand, similar experiments on cats gave contradictory results. In these animals the blood pressure usually sank only to 70 mm., seldom to less than 45 mm. of mercury. Under these conditions very distinct precipitates and a decided vital staining of the epithelium were observed, and yet the excretion of carmine in the urine was very small. When the blood pressure in the cats, after section of the spinal cord, was lowered still further by means of amyl nitrate and other agents to as low as 20 mm. of mercury, the dyestuff precipitation and the granular staining of the epithelial cells failed to occur.

We lay great stress upon these experiments dealing with artificially lowered blood pressure because one would anticipate that filtration through the glomeruli would be more easily influenced by blood pressure variations than would secretion through the epithelium. But since, in addition, an adequate lowering of the blood pressure results in an absence of vital staining in the primary tubules, one is justified in concluding that the epithelium is not concerned in the excretion of dyes.

Naturally, such conclusions must be confirmed by further evidence. For this purpose, Mitamura employed the old Nussbaum preparation of the frog's kidney. As is well known, the glomeruli of the frog's kidney are supplied by the renal artery, whereas the primary tubules are supplied by a special vessel, a renal branch of the portal vein. One can, therefore, assume that tying off the renal artery would interfere with the function of the glomeruli, but not of the tubules. Similar experiments have been performed by numerous other investigators but have not led to any uniform results. The chief difficulty has always been that anastomoses exist between the arterial system of the glomeruli and the venous supply of the tubules, so that the passage of some blood from one to the other could not be entirely avoided. There

is another great objection to all experiments in which an attempt is made to study the function of the tubules by tying off the renal artery, leaving intact the blood supply via the renal branch of the portal vein. It could be said that by cutting off the stream of filtrate from the glomeruli one also interferes with the secretion from the tubules, for the tubules are thereby deprived of a fluid solvent for the secreted masses of material.

Mitamura tried to overcome these difficulties and objections, first, by perfusing each of the vascular systems with different fluids and second, by simultaneously varying the pressure within them. If the renal artery is tied off on one kidney and the animal subsequently injected with carmine, the operated kidney will show no carmine deposits within the tubules and no vital staining. It is an easy matter to demonstrate that blood from the portal vein reaches the glomeruli through the anatomoses. If in spite of this no excretion of carmine occurs, it may only be due to the fact that the blood pressure in the portal vein is too low and is therefore inadequate to produce a filtration of urine.

In order to confirm this assumption, the renal artery and the renal branch of the portal were simultaneously perfused with Ringer's solution of a pH. concentration of 7.6 to 7.7. Under such conditions, the kidneys will retain their normal mitochondria structure for at least two hours. The carmine was employed as a 0.04 per cent. solution in this Ringer's fluid. Usually the urine was collected by means of a bladder catheter and the drops counted. The normal rate of urinary flow was about one drop every ten minutes.

The results were as follows:

1. Normal arterial and normal venous pressure; perfusion of the artery with carmine, and of the vein with plain Ringer's solution; result, always an excretion of carmine in a concentration equivalent to that of the perfusion fluid.

2. Normal arterial and normal venous pressure; perfusion of the vein with carmine and of the artery with plain Ringer's solution; result, never any excretion of carmine in spite of the fact that the volume of urinary flow remained normal.

3. Normal arterial pressure but hypertension in the vein; perfusion of the vein with carmine and of the artery with plain Ringer's solution; result, extremely variable.

4. Arterial hypotension and venous hypertension; perfusion of the vein with carmine and of the artery with plain Ringer's solution; result, usually no urinary flow and no excretion of carmine.

In so far as the staining of the epithelium is concerned, it is in similar experiments only found in those cases where an excretion of the dye in the urine is also observed.

One can positively conclude from the experiments of series 1 and 2 that carmine is only excreted through the glomeruli and that the primary convoluted tubules are not concerned in the process. As far as the excretion of carmine is concerned, the theory of a secretion through the tubules must be abandoned. Series 3 indicates that anastomoses must exist between the renal branch of the aortal vein and the renal artery. Series 4 proves that venous hypertension is unable to compensate for an arterial hypotension, and cannot result in any filtration.

Mitamura's experiments present both morphologic and physiologic proof that the excretion of vital dyes, or at least of carmine, takes place exclusively through the glomeruli. This excretion occurs in extremely slow concentration. But as a result of resorption of water within the primary tubules, there occurs a concentration of the urine and a resultant precipitation of carmine masses in the region of the marginal membrane. The resorbed fluids carry some dye with them into the epithelium of the primary tubules, and in this fashion there occurs the vital staining; in other words, a condensation of the resorbed dye into granular particles.

We shall henceforth designate the glomeruli as the filtration apparatus and the primary convoluted tubules as the resorption apparatus. I am therefore obliged to change the opinion I expressed ten years ago that the depositions within the primary tubules are evidence of a secretory activity on the part of these cells. If, on the basis of Ludwig-Cushny's theory and of

Mitamura's observations, we must now consider Suzuki's deposition phenomenon as the result of a resorptive process, the question then arises, what rôle does the third important apparatus in the kidney, the system of loops, play?

As already mentioned, we find numerous casts at a certain period of dye excretion, and for this reason we originally named this part of the tubular labyrinth, the resorptive portion. But if resorption is chiefly confined to the primary tubules—a fact also attested to by the presence of the unique marginal membrane—it would seem quite improbable that the system of loops which possess a totally different structure should exert the identical function. One must rather think of some other purpose for them. At any rate, our observations are solely confined to the thin limbs of the loops, for here the peculiar casts are found. Similarly, in human pathology, casts are regularly found obstructing the curved ends of the loops. Here, then, must be a point of predilection. But how could casts form at this site, unless an inspissation, a resorptive process took place? Mitamura believes that these casts form in the thin limbs essentially as a result of precipitation which occurs in the primary tubules, but which are gradually carried downward into the loops by the urinary stream. The loops are placed like a syphon between primary tubules and intercalary tubules. It is permissible to surmise that the system of loops acts as a sort of pressure regulator which perhaps has some relationship to the pressure in the vascular system. But these are only guesses which need careful experimental study and a resorption process of quite another nature, as in the primary convoluted tubules, cannot be excluded.

With regard to the function of Henle's loops, certain observations made by American and German investigators appear exceedingly important. The primary tubules of every glomerulus are of equal length; on the other hand, the loops are of variable lengths. This would indicate that the filtration and resorption apparatus are exactly balanced. The system of loops, however, must exert some common influence on the two other systems. And yet the length of the loops must also be dependent upon

some other factors, such perhaps, as the position of the glomerulus within the cortex.

In view of this newer conception that a tubular labyrinth is composed of a filtration system, a resorption system and a pressure regulating system, we must alter our conceptions concerning renal disease. We must first attempt to explain the action of certain parenchymatous poisons upon the tubules. We know as a result of Suzuki's investigations that the important parenchymatous poisons damage the primary tubules and, in fact, pick out different parts of it. Chromium chiefly damages the first portion, uranium the third portion and sublimate and cantharidin the terminal transition piece. How can this be explained? If indeed the excretion of all these poisons took place exclusively in the glomeruli and only a resorption occurred in the primary tubules, one would naturally expect that all of them ought to attack the first portion with greatest intensity, the portion in which the vital staining is most distinct. But, on the contrary, as we have seen, these poisons attack entirely different sites. This can only be explained as follows: that the structure of the epithelium and of its marginal membrane varies in the different portions of the primary tubule, and that the resorption of molecules of different sizes and of various colloidal suspensions occurs at different rates. But unfortunately, we know nothing about the manner in which the various poisons occur in solution in the provisional urine, so that we are unable to express an opinion on this important problem. Only one thing must be mentioned. Endogenously formed substances are also excreted through the glomeruli and then resorbed by the epithelium of the primary tubules where they are often stored—as for example, the yellow iron salts in hemosiderosis, the green bile pigments, the reddish-brown hemoglobin. All of these substances are deposited most markedly in the proximal portion of the primary tubules.

On the other hand, this is not the case with glycogen. Doctor Baehr has been able to demonstrate that in human beings with diabetes a precipitation of glycogen occurs in the marginal mem-

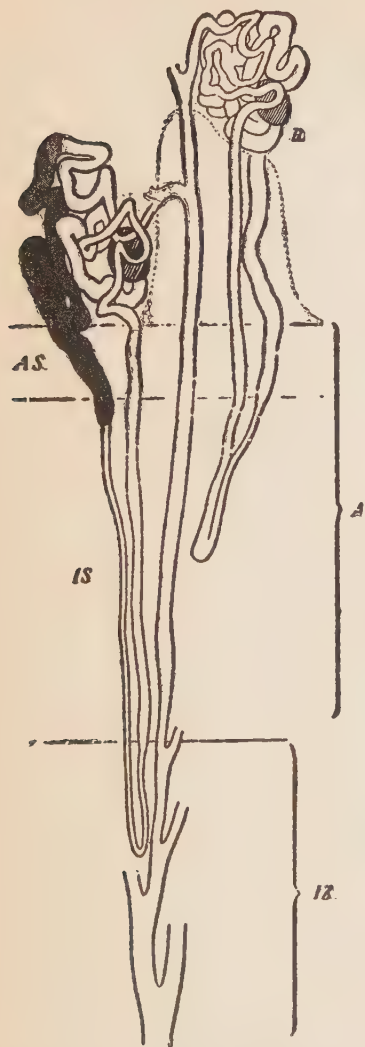


FIG. 3.—Localization of the epithelial damage in the convoluted tubule after injection of cantharidin. (After Suzuki).

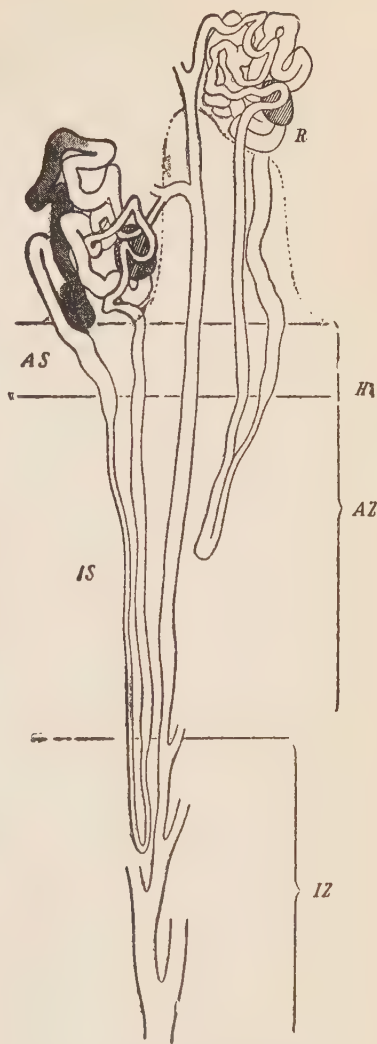


FIG. 4.—Localization of the epithelial damage in the convoluted tubule after injection of chromium. (After Suzuki).

brane, exactly duplicating the picture seen in the carmine animals. This would indicate that here there occurs a resorption of glycogen from the glomerular filtrate. But, unlike the observations made on the carmine animals, the intracellular deposition of glycogen occurs in the terminal straight transition portion and not in the proximal parts of the convoluted tubules. We are still entirely at a loss for an explanation of this phenomenon. Future investigations must concern themselves with a solution of this problem concerning the various localization of resorbed substances in different parts of the primary tubules and especially with a reason for the localization of the effects of different poisons.

In view of the fact that parenchymatous, that is, tubular, injury to the kidney produced by toxins and poisons will play a very great rôle in such studies, I believe it now desirable to touch upon another question, namely, the fact that renal epithelium seems to be able to develop some immunity, or at least increased resistance, to such poisons. Suzuki called attention to the fact that animals which had been poisoned with uranium and had recovered, could later withstand the re-injection of what would otherwise have been lethal doses. Examination of the kidneys in the course of these experiments revealed a surprising observation. The *renal epithelium*, which ordinarily would have been severely damaged by such large doses, was perfectly well preserved. This seemed to indicate that it might be possible to induce a certain amount of immunity of renal epithelium to such poisons. Personally, I consider this particular question of extreme importance because upon it must hinge our conception of parenchymatous nephritis.

According to Metchnikoff, Adami and others, an essential characteristic of inflammatory processes consists in the fact that it essentially represents a defense mechanism. In the case of infections, this defense mechanism is, as a rule, associated with a process of immunization. If a tissue or a cell develops an increased resistance against a bacterial or other poison, it is safe to assume that these particular cells have taken part in the proc-

ess of defense. I am therefore prepared to express the belief that parenchymatous or tubular nephritis represents a reaction of the kidney to some poison, and that in the case of this reaction the epithelium itself takes part in the chemical transformation of the poison and in its detoxication. The kidneys of an animal which has survived a parenchymatous, 1:2, a tubular, nephritis, produced by a poison, must show an increased resistance of the epithelium to further administration of the same poison.

Such observations have actually been made in the course of investigations by Doctor Gil-y-Gil. Animals which had survived a single injection of 0.01 gms. of uranium could be given eighty times the dose subcutaneously or ten times intravenously without the development of any evidences of poisoning, provided that in interim the animal had received gradually increasing doses. It was possible to ascertain by means of urinary examinations that during the course of these immunization experiments the uranium was not retained in the body, but was actually excreted through the kidneys. In other words, the uranium was able to pass through the kidneys of an immunized animal without damaging them. This can only be due to the development of a local immunity of the renal epithelium.

Another interesting fact revealed by some of these experiments was that just those portions of the primary convoluted tubules which ordinarily are damaged by the first injection of uranium (the middle and distal portions) are entirely unaffected by subsequent injections of uranium, whereas the proximal portions now present the most marked changes. On the other hand, instances occurred in which repeated injections of large doses damaged the epithelium of the primary tubules most severely but did not produce the glomerular changes which were produced by Baehr with uranium. Here again it is necessary to consider the possibility of an immunity of the glomeruli.

Injection experiments on such acutely or chronically poisoned animals whose kidneys had developed more or less of an immunity, demonstrated also that the injury produced was not distributed uniformly throughout the kidneys. The damage

appeared to have occurred rather in small spotted areas, so that some of the glomeruli still remained permeable to the dye, whereas others were entirely impermeable. Just those tubules which belong to the permeable glomeruli presented the severer types of epithelial damage. It is therefore necessary to assume that the poison is essentially excreted through the glomeruli, and only damages the epithelium on being reabsorbed from the lumen of the tubules. These observations are entirely in accord with Cushny's theory and with the anatomical findings of Mitamura. Pathological findings speak therefore in favor of a close relationship between glomerulus and primary tubules, since together they represent a unified filtration and resorption apparatus. We must therefore re-analyze all renal function tests in the light of this new viewpoint. It is impossible for me to enter into a consideration of all the experiments of this character, such as those using the various methods by Schleyer. The division of all nephritis into vascular and tubular varieties which he advocated, has found ample critical refutation in the work of Suzuki and more recently in the publications of Cushny, and yet I would like to describe very briefly some new experiments upon the excretory ability of the kidney made by means of determinations of the hydrogen-ion concentration of the urine.

We know that the acid or alkaline reaction of the urine is dependent upon the food and the metabolism of the body, and can relatively easily be varied from the acid to the alkaline side. The question then arises, how does this ability to excrete more or less acid or base influence diseases of the kidney? The experimental observations of Rehn and Mitamura would lead us to believe that all glomerular diseases are favored by a high acid concentration of the urine, whereas tubular damage is favored by an increased alkalinity. These observations were correct whenever the functional test was made by administration of hydrochloric acid or sodium bicarbonate. These observations have been repeatedly confirmed both experimentally and clinically, but we are still unable to explain them with any degree of assurance. If we assume that a resorption of the "threshold "

salts occurs in the region of the primary tubules, it is easy to understand that in the presence of a marked tubular damage such resorption might not occur; for as a matter of fact just those salts which are responsible for the alkalinity of the urine do not appear to be resorbed.

I am, therefore, able to conclude my report upon these newer investigations of the filtration and secretion theories as far as they have been carried from a morphological standpoint. I am well aware that in this country the problem is being attacked most industriously. These functional tests represent a very important and welcome addition clinically, to the purely physiological and histological investigations of the kidney. They afford the possibility of separating, more sharply, clinically also, the diseases of the filtration apparatus from those of the resorption apparatus. It is well known that the question whether there is in man a purely tubular-inflammatory disease of the kidneys in the sense of a defensive reaction, and whether such tubular or pharenchymatous nephritides may lead to similar forms of contraction, such as the much more frequent glomerular nephritides, is as yet not solved. I am, however, convinced that such tubular nephritides do occur in man, that is, such changes as can be produced experimentally with small doses of uranium, chromium, submese, etc. Such tubular changes can and undoubtedly do lead to some contraction of the kidney. I therefore must agree to the existence of pure tubular contracted kidneys as described by Ophüls and Dickson. How often they occur in human beings it is extremely difficult to say in view of the fact that it is almost impossible to recognize a primary tubular damage when the process has reached the stage of a markedly contracted kidney. However, our investigations in chronic uranium poisoning have revealed exquisite examples of this type of contracted kidney. In view of the fact that we now have this experimental method of producing contracted kidneys, one ought to study more carefully the effect of such contracted kidneys upon the heart and the vascular system. We may, therefore, hope that the better knowledge of the function of the individual

portions of the kidney system and the possibility now furnished of testing the function of these individual portions on the living body, will lead to a sharper delimitation of the diseases of the kidney, and especially to the recognition of the clinically interesting, though rare, parenchymatous nephritis and its contraction form.

BIBLIOGRAPHY

Suzuki, T., Nierensekretion, G., Fischer, Jena: 1912.

Cushny, A. R.: The Secretion of Urine, London, 1917.

Møllendorff: Vitale Färbungen an tierischen zellen. *Ergebn. d. Physiol.*, 1920, xviii.

Mitamura: Filtration-Resorptions Prozesse i. d. Niere (to be published by Fisher, Jena).

Gil-y-Gil, C.: Die Immunität im Nierenepithelgewebe. *Ziegler's Beiträge*, 1924, Bd. lxxii.

~~8115~~



